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                   data from INPADOC
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 NEWS  13 MAR 22   REGISTRY/ZREGISTRY enhanced with experimental property tags
 NEWS  14 APR 04   EPFULL enhanced with additional patent information and new
                   fields
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                   may be affected by a change in filing date for U.S.
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          7774 PARAMETERS
          9174 PARAMETER
                 (PARAMETER OR PARAMETERS)
         40735 PRODUCT
         28323 PRODUCTS
         61034 PRODUCT
                 (PRODUCT OR PRODUCTS)
L1          85 (PARAMETER (3A) PRODUCT)

=> s l1 and (weight (3a) property)
         10971 WEIGHT
           431 WEIGHTS
         11237 WEIGHT
                 (WEIGHT OR WEIGHTS)
          2333 PROPERTY
         18591 PROPERTIES
         20334 PROPERTY
                 (PROPERTY OR PROPERTIES)
            35 WEIGHT (3A) PROPERTY
L2           0 L1 AND (WEIGHT (3A) PROPERTY)

=> s l1 and py<2000
        248714 PY<2000
                 (PY<2000)
L3          63 L1 AND PY<2000

=> d 1-10 bib ab

L3    ANSWER 1 OF 63  BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
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AN 1999-11084 BIOTECHDS
TI Biotechnology for the production of nutraceuticals enriched in conjugated linoleic acid: I. Uniresponse kinetics of the hydrolysis of corn oil by a Pseudomonas sp. lipase;
immobilized in a hollow fiber reactor; mathematical model
AU Sehanputri P S; *Hill Jr C G
CS Univ.Wisconsin-Madison
LO Department of Chemical Engineering, University of Wisconsin-Madison, 1415 Engineering Drive, Madison, WI 53706, USA.
Email: hill@engr.wisc.edu
SO Biotechnol.Bioeng.; (**1999**) 64, 5, 568-79
CODEN: BIBIAU ISSN: 0006-3592
DT Journal
LA English
AB The kinetics of corn oil hydrolysis in the presence of a lipase (EC-3.1.1.3) from Pseudomonas sp. immobilized within the walls of a hollow fiber fermentor can be modeled in terms of a three-parameter rate expression. This rate expression consists of the **product** of a two-**parameter** rate expression for the hydrolysis reaction itself (which is of the general Michaelis-Menten form) and a first-order rate expression for deactivation of the enzyme. Optimum operating conditions correspond to 30 deg and buffer pH of 7 during both immobilization of the enzyme and the hydrolysis reaction. Under these conditions, the total fatty acid concentration in the effluent oil stream for a fluid residence time of 4 hr was approximately 1.6 M. This concentration corresponds to hydrolysis of about 50% of the glyceride bonds present in the feedstock corn oil. The fatty acid of primary interest in the effluent stream is linoleic acid. (26 ref)

L3 ANSWER 2 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1999-08921 BIOTECHDS
TI Kinetic modeling of omega-transamination for enzymatic kinetic resolution of alpha-methylbenzylamine;
omega-transaminase production from Bacillus thuringiensis for application in racemate resolution; a kinetic model
AU Shin J S; *Kim B G
CS Univ.Seoul-Nat.Inst.Mol.Biol.Genet.
LO Division of Chemical Engineering and Institute for Molecular Biology and Genetics, Seoul National University, 151-742, Seoul, Korea.
Email: byungkim@plaza.snu.ac.kr
SO Biotechnol.Bioeng.; (**1998**) 60, 5, 534-40
CODEN: BIBIAU ISSN: 0006-3592
DT Journal
LA English
AB Omega-transaminase was produced from Bacillus thuringiensis JS64 following fermentation (pH 7) in a fermentor at 37 deg for 14 hr with 500 rpm. A kinetic model for the enzyme was developed using the King-Altman method to simulate the kinetic resolution of alpha-methylbenzylamine (I). Starting from a ping-pong bi-bi mechanism, a complete kinetic model including substrate inhibition only in the reverse reaction was developed. The asymmetric synthesis of (S)-(I) proved to be difficult due to a much lower maximum reverse reaction rate than the maximum forward reaction rate, thermodynamically exergonic forward reaction (i.e. transamination between (S)-(I) and pyruvate), and the severe product and substrate inhibition of the reverse reaction. Experimental values for kinetic **parameters** showed that the **product** inhibition constant of (S)-(I) is the most important parameter on determining the resolution reaction rate, suggesting that the resolution reaction rate will be very low unless (S)-(I) strongly inhibits the reverse reaction. Using the kinetic model, the resolution of (I) in aqueous buffer was simulated. (25 ref)

L3 ANSWER 3 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1999-04912 BIOTECHDS

TI Optimization of temperature and initial pH and kinetic analysis of tartaric acid production by Gluconobacter suboxydans; for use as flavor enhancer, preservative, antioxidant in oil and in drug delivery

AU Chandrashekar K; Felse P A; *Panda T

CS Indian-Inst.Technol.Madras

LO Biotechnology Research Centre, Department of Chemical Engineering, Indian Institute of Technology, Madras, Chennai 600 036, India.

SO Bioprocess Eng.; (**1999**) 20, 3, 203-07
CODEN: BIENEU ISSN: 0178-515X

DT Journal

LA English

AB Response surface methodology was applied for the simultaneous optimization of initial pH and temperature for the production of tartaric acid by Gluconobacter suboxydans NCIM 2049. A logistical kinetic model was formulated for growth of G. suboxydans. The culture medium contained (kg/cu m) 20 sorbitol and 2 yeast extract. Batch culture was carried out in a stirred tank fermentor. A temperature of 32.8 deg and an initial pH of 6.05 was found to be the most suitable. The Leudeking-Piret model explained the **product** formation. **Parameters** of the model were evaluated and tartaric acid formation was found to be non-growth associated. Tartaric acid is used in the acidification of wine malts, as a flavor enhancer and as a preservative in fóod products. It is also used as an antioxidant in oils and as a carrier for many drugs. (12 ref)

L3 ANSWER 4 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

AN 1997-07240 BIOTECHDS

TI The use of normal diploid cells for production of therapeutic agents; diploid cell culture characterization for vaccine, therapeutic agent and somatic cell therapy (conference abstract)

AU Ryan J M

CS Abbott-Lab.

LO D-456, Abbott Laboratories Inc., North Chicago, IL 60064, USA.

SO In Vitro; (**1997**) 33, 3, Pt.2, V-5
CODEN: ITCSAF ISSN: 0883-8364
In Vitro Biology Congress, Society for In Vitro Biology 1997 Meeting, Washington, DC, 14-18 June, 1997.

DT Journal

LA English

AB Normal diploid cell cultures are used for the production of vaccines, therapeutic agents and somatic cell therapies. Characterization should be a continuous process that starts with donor testing, as related to their health and the microbial, parasitic and viral makeup of the locale, through to final product formation. Cell characterization should include donor information, definition of culture establishment procedures and details regarding formation of master and working cell banks. cells should be characterized during serial propagation and **product** formation. **Parameters** evaluated should include, but not be limited to, establishment that the donor was free of obvious microbial and viral infection, determination of culture identity and normalcy and verification of the absence of viral, mycoplasma and microbial contamination. Culture performance qualifications should be demonstrated by reproducible cell proliferation, product formation and quality characteristics. A model system will be used to illustrate the above approach for using normal diploid cells for product formation. (0 ref)

L3 ANSWER 5 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

AN 1997-03754 BIOTECHDS

TI Characterization of monoclonal IgA production and activity in hollow fiber and fluidized bed reactors; Vibrio cholerae lipopolysaccharide monoclonal antibody IgA preparation by mouse hybridoma cell culture using protein-free culture medium in hollow fiber membrane culture vessel (conference paper)

AU Stoll T S; Ruffieux P A; Lullau E; von Stockar U; Marison I W
CS Inst.Chem.Eng.Lausanne; Swiss-Fed.Inst.Technol.
LO Cell Culture and Fermentation R&D, Genentech, Inc., 460 Point San Bruno Blvd, South San Francisco, CA 94080-4990, USA.
SO Prog.Biotechnol.; (**1996**) 11, 608-14
CODEN: PBITE3 ISSN: 0921-0423
Immobilized Cells: Basics and Applications, Noordwijkerhout, The Netherlands, 26-29 November, 1995.
DT Journal
LA English
AB A mouse hybridoma cell culture, Zac3, secreting an IgA of allotype 2 directed against the surface lipopolysaccharide of Vibrio cholerae, was cultured in serum-containing culture medium and a protein-free culture medium in three different culture vessels: a small hollow fiber membrane culture vessel; a larger hollow fiber membrane culture vessel; and a fluidized bed culture vessel. A wide range of values for the active fraction of IgA was observed in the hollow fiber membrane culture vessels; this **parameter** varying with the **product** concentration, with the lowest numbers corresponding to the lowest IgA concentrations (2 g/l of less). Western blot analyses revealed that IgA produced at high concentration contained important fractions of polymers and aggregates. In addition, the active fraction of IgA was higher in the protein-free culture medium. Further assays, such as glycosylation studies, are required for further comparisons of production systems. (5 ref)

L3 ANSWER 6 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1996-11099 BIOTECHDS
TI Production of L(+) and D(-) lactic acid isomers by Lactobacillus casei subsp. casei DSM 20011 and Lactobacillus coryniformis subsp. torquens DSM 20004 in continuous fermentation;
lactic acid bacterium continuous culture; application in biomedical industry
AU Gonzalez-Vara y R A; Pinelli D; Rossi M; Fajner D; Magelli F; *Matteuzzi D
CS Univ.Bologna
LO C.I.R.B.-Centre for Biotechnology, University of Bologna, Department of Pharmaceutical Sciences, via Belmeloro 6, 40126 Bologna, Italy.
SO J.Ferment.Bioeng.; (**1996**) 81, 6, 548-52
CODEN: JFBIEX ISSN: 0922-338X
DT Journal
LA English
AB L(+) and D(-) lactic acid isomer production by Lactobacillus casei subsp. casei DSM 20011 and Lactobacillus coryniformis subsp. torquens DSM 20004 was investigated under continuous culture conditions. A fermentor (2 l working volume) was inoculated (10% v/v) with a 16 hr culture and fermentation was carried out at 36 deg with 120 rpm. Experiments were performed in MRS broth and slightly modified Murashige-Skoog culture medium containing (g/l) 40 glucose, 30 yeast extract, 0.6 $MgSO_4.7H_2O$, 1 sodium acetate, 0.03 $FeSO_4.7H_2O$, 0.03 $MNSO_4.H_2O$, 0.5 KH_2PO_4 and 0.5 K_2HPO_4 (pH 6.5). Lactic acid concentrations were determined by HPLC. Results showed that the dilution rate influenced the fermentation pattern, modifying various fermentation **parameters**. Nevertheless, the **product** and biomass yields remained constant and the ratio of the L(+) and D(-) lactic acid isomers was not affected by the dilution rate. The optimum glucose concentration on inlet feed medium was also determined for the L. coryniformis fermentation. L(+) and D(-) lactic acid may be used in poly-lactic acid polymer production for biomedical applications. (18 ref)

L3 ANSWER 7 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1996-08424 BIOTECHDS
TI Reaction mechanism of a new glycosyltrehalose-producing enzyme isolated from the hyperthermophilic archaeum, Sulfolobus solfataricus KM1;

potential trehalose production using thermostable glycosyltransferase and amylase produced by archaebacterium

AU Kato M; Miura Y; Kettoku M; Komeda T; Iwamatsu A; *Kobayashi K

CS Kirin-Brew.

LO Applied Bioresearch Center, Kirin Brewery Co., Ltd., 3 Miyaharacho, Takasakishi, Gunma 370-12, Japan.

SO Biosci.Biotechnol.Biochem.; (**1996**) 60, 5, 925-28
CODEN: BBBIEJ ISSN: 0916-8451

DT Journal

LA English

AB An amylolytic activity, capable of catalyzing the conversion of soluble starch into alpha,alpha-trehalose was found in a cell homogenate of the hyperthermophilic acidophilic archaeum Sulfolobus solfataricus KM1 (grown aerobically at 75 deg in Brock medium (pH 3)). A glycosyltransferase and an amylase contributing to this activity, were purified to homogeneity. The amylase was found to be an alpha-amylase (EC-3.2.1.1) and had maximal activity at pH 4.5-5.5 and at 70-85 deg. Almost 100% of the activity remained even after incubation of the enzyme for 6 hr at 85 deg. The alpha-amylase catalyzed the hydrolysis of glycosyltrehaloses to trehalose. Based on a detailed analysis of the reaction **products**, kinetic **parameters** and an experiment using 3H-labeled substrates, it was shown that the alpha-amylase hydrolyzed only the alpha-1,4-glucosidic bond adjacent to the trehalose unit of the glycosyltrehaloses. 6 Strains of the Sulfolobaceae family were shown to have the alpha-amylase. (15 ref)

L3 ANSWER 8 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

AN 1996-08423 BIOTECHDS

TI Reaction mechanism of a new glycosyltrehalose-producing enzyme isolated from the hyperthermophilic archaeum, Sulfolobus solfataricus KM1; potential trehalose production using thermostable glycosyltransferase and amylase produced by archaebacterium

AU Kato M; Miura Y; Kettoku M; Shindo K; Iwamatsu A; *Kobayashi K

CS Kirin-Brew.

LO Applied Bioresearch Center, Kirin Brewery Co., Ltd., 3 Miyaharacho, Takasakishi, Gunma 370-12, Japan.

SO Biosci.Biotechnol.Biochem.; (**1996**) 60, 5, 921-24
CODEN: BBBIEJ ISSN: 0916-8451

DT Journal

LA English

AB Amylolytic activity, capable of catalyzing the conversion of soluble starch to alpha,alpha-trehalose was found in a cell homogenate of hyperthermophilic acidophilic archaeum Sulfolobus solfataricus KM1 (grown aerobically at 75 deg in Brock medium (pH 3)). A glycosyltransferase (GTase) and an alpha-amylase (EC-3.2.1.1) contributing to this activity, were purified to homogeneity. The GTase was optimally active at pH 5.0-6.0 and at 70-80 deg, and retained 91% activity after incubation for 6 hr at 85 deg. A GTase catalyzed the conversion of malto-oligosaccharides to glycosyltrehaloses. Based on a detailed analysis of the reaction **products**, kinetic **parameters** and an experiment using 3H-labeled substrates, it was shown that GTase transferred an oligomer segment of malto-oligosaccharide to the C1-OH position of glucose, located at the reducing end of the malto-oligosaccharide, to produce a glycosyltrehalose with an alpha-1,1 linkage. The reaction was intramolecular. 9 Strains of the Sulfolobaceae family were shown to have GTase. (15 ref)

L3 ANSWER 9 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

AN 1996-02769 BIOTECHDS

TI Comparison of 14 cationic liposomes transfection efficiency in hematopoietic cells; lipofection-mediated beta-galactosidase reporter gene transfer and expression in hematopoietic stem cell culture for gene therapy (conference abstract)

AU Floch V; Le Bolc'h G; Clement J C; Yaouanc J J; Audrezet M P; Mercier B; des Abbayes H; Abgrall J F; Ferec C
CS Univ.Brest; CNRS
LO Centre de Biogenetique, Universite Brest, E.T.S.B.O., BP 454, 29275, Brest Cedex, France.
SO Gene Ther.; (**1995**) 2, Suppl.1, S8
CODEN: 4352W ISSN: 0969-7128
Human Gene Transfer and Therapy, 3rd Meeting, Barcelona, Spain, 17-20 November, 1995.
DT Journal
LA English
AB Transfection of hematopoietic cells with non-viral vectors is an important challenge for gene therapy project involving hematopoietic cells in inherited diseases as well as acquired diseases. Twelve new lipid/DNA complexes were targeted at hematopoietic stem cells (K562) and compared using a quantitative assay, which permitted an estimation of the quantity of beta-galactosidase (EC-3.2.1.23) produced. The 12 novel molecules were obtained by the Mannich reaction of lipophilic dialkylphosphites with secondary amines and subsequent quaternarization. All the new complexes were tested at different DNA/lipid ratio with and without 50% DOPE and 3 were at least as efficient as the commercially available lipids DOTMA:DOPE and DOSPA:DOPE. The difference in formulations of these **products** allowed the **parameters** which influence the efficiency of transfection in hematopoietic cells to be analyzed and the observations were used to increase the efficiency of the products and to decrease the toxicity of the vectors which could be good candidates for gene transfer protocol in hematology. (0 ref)

L3 ANSWER 10 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1995-15424 BIOTECHDS
TI Effect of cell concentration on the kinetics of whole-cell enzyme entrapped within calcium alginate;
Trichoderma reesei beta-glucosidase immobilization and characterization
AU Lee W C; Lee R Y; Ruaan R C
CS Univ.Chung-Cheng-Nat.; Univ.Chung-Yuan-Nat.
LO Department of Chemical Engineering, National Chung Cheng University, Chia-Yi, 621 Taiwan, Republic of China.
SO Biotechnol.Prog.; (**1995**) 11, 4, 461-64
CODEN: BIPRET ISSN: 8756-7938
DT Journal
LA English
AB A method was presented for determining the kinetics of whole-cell enzymes entrapped within calcium alginate gel beads, using Trichoderma reesei QM 9414 beta-glucosidase (EC-3.2.1.21) Ca-alginate gel beads suspended in a 250 ml flask with 100 ml of citrate buffered (0.05 M, pH 4.8) cellobiose solution. The flask was shaken at 50 deg and 250 rpm, and glucose produced as a result of cellobiose hydrolysis was measured. Substrate and **product** diffusivities and kinetic **parameters**, i.e. the Michaelis constant and the maximum velocity, could be estimated by fitting experimental data to a proposed mathematical model. The immobilized cell concentration was the main factor causing a decrease in maximum velocity. The influence of cell concentration in the gel beads on the variation in the maximum velocity could be isolated from the effect of cell concentration of diffusional limitation and evaluated separately. The whole-cell enzymes became partially difficult to access by substrate when the cell population increased. This resulted in a decrease in the maximum velocity and apparent initial velocity. (20 ref)

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FILE 'BIOTECHDS' ENTERED AT 13:52:37 ON 13 MAY 2005
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L2 0 S L1 AND (WEIGHT (3A) PROPERTY)
L3 63 S L1 AND PY<2000

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L4 ANSWER 11 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1995-14971 BIOTECHDS
TI Bubble free gaseous transfer in bioreactors using perfluorocarbons; Escherichia coli chemostat culture
AU Martin S; Soucaille P; Condoret J S
CS INSA
LO Institut National des Sciences Appliquees, Centre de Bioingenierie Gilbert Durand, Complexe Scientifique de Rangueil, 31077 Toulouse Cedex, France.
SO Bioprocess Eng.; (**1995**) 13, 6, 293-300
CODEN: BIENEU ISSN: 0178-515X
DT Journal
LA English
AB Bubble-free oxygenation was used to ensure oxygen supply and carbon dioxide extraction in an Escherichia coli chemostat culture. The culture was operated at a dilution rate of 0.275/hr under atmospheric pressure at 35.5 deg. Foralkyl, a perfluorocarbon, added in an emulsion to the influent culture medium at 50% volume, provided 0.17 g O2/l.hr and extracted 0.23 g CO2/l.hr for a culture at 0.74 g/l biomass. This oxygen supply was close to maximum theoretical oxygenation by Foralkyl at this pressure, when imposing a minimum oxygen concentration of 1 mg/l in the water phase. Quantification of oxygen transfer was not done by direct measurement of oxygen transfer rates, since conventional oxygen concentration measurement by membrane polarography probe in an emulsion was unreliable. Evaluation was carried out by referring to conventional aerated culture, whose measurable **parameters** (biomass and **product** concentrations) were unaffected when shifting to this novel aeration system. (19 ref)

L4 ANSWER 12 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1995-08758 BIOTECHDS
TI Steady-state kinetics of electrochemical polyenzyme membrane sensors (a review); amperometric biosensor constructed from an immobilized enzyme
AU Sorochinskii V V; Kurganov B I
CS Russian-Acad.Sci.Bakh-Inst.Biochem.
LO Bakh Institute of Biochemistry, Russian Academy of Sciences, Leninskii pr. 33, Moscow, 117071 Russia.
SO Prikl.Biokhim.Mikrobiol.; (**1995**) 31, 3, 283-95
CODEN: PBMIAK ISSN: 0555-1099
DT Journal
LA Russian
AB The results of investigations of steady-state kinetics of electrochemical membrane sensors involving polyenzymatic conversions of the assayed substrate are summarized. The analytical expressions for operational **parameters** of **product**-sensitive sensors with uniform or laminated distribution of immobilized enzymes in the coating are presented. The dependences of the sensitivity of amperometric bienzyme sensors on physicochemical characteristics of the coating and on the presence of inhibitors was analyzed. (38 ref)

L4 ANSWER 13 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1995-13391 BIOTECHDS
TI Influence of bioprocess conditions on glycoform patterns of recombinant glycoproteins;
bioprocess condition and glycoform pattern of recombinant glycoprotein in recombinant Chinese hamster ovary continuous cell culture (conference abstract)
AU Schuppenhauer M R; Smala A M; Dunn I J
CS Swiss-Fed.Inst.Technol.
LO Department of Chemical Engineering, ETH Zurich, CH-8092 Zurich, Switzerland.
SO Abstr.Pap.Am.Chem.Soc.; (**1995**) 209 Meet., Pt.1, BIOT025
CODEN: ACSRAL ISSN: 0065-7727
209th ACS National Meeting, Anaheim, CA, 2-6 April, 1995.
DT Journal
LA English
AB Long-term productivity and product quality were assessed during continuous cultivation of a recombinant CHO cell line in a fluidized bed culture vessel. Application of step changes for the dilution rate, glutamine and glucose (as lead substrate) gave dynamic responses for metabolic and **product parameters**. The application of various electro-focusing and blotting protocols indicated consistent patterns for several glycoforms. Switching to a proprietary serum-free formulation induced a 3-fold production increase paralleled by a more pronounced glycoform diversity in the upper and lower pI range. These results suggest that changing environmental conditions may alter the metabolic pathway and biosynthesis and influence the post-translational modification. Previous results for high density systems may be explained by these findings. (0 ref)

L4 ANSWER 14 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1996-02769 BIOTECHDS
TI Comparison of 14 cationic liposomes transfection efficiency in hematopoietic cells;
lipofection-mediated beta-galactosidase reporter gene transfer and expression in hematopoietic stem cell culture for gene therapy (conference abstract)
AU Floch V; Le Bolc'h G; Clement J C; Yaouanc J J; Audrezet M P; Mercier B; des Abbayes H; Abgrall J F; Ferec C
CS Univ.Brest; CNRS
LO Centre de Biogenetique, Universite Brest, E.T.S.B.O., BP 454, 29275, Brest Cedex, France.
SO Gene Ther.; (**1995**) 2, Suppl.1, S8
CODEN: 4352W ISSN: 0969-7128
Human Gene Transfer and Therapy, 3rd Meeting, Barcelona, Spain, 17-20 November, 1995.
DT Journal
LA English
AB Transfection of hematopoietic cells with non-viral vectors is an important challenge for gene therapy project involving hematopoietic cells in inherited diseases as well as acquired diseases. Twelve new lipid/DNA complexes were targeted at hematopoietic stem cells (K562) and compared using a quantitative assay, which permitted an estimation of the quantity of beta-galactosidase (EC-3.2.1.23) produced. The 12 novel molecules were obtained by the Mannich reaction of lipophilic dialkylphosphites with secondary amines and subsequent quaternarization. All the new complexes were tested at different DNA/lipid ratio with and without 50% DOPE and 3 were at least as efficient as the commercially available lipids DOTMA:DOPE and DOSPA:DOPE. The difference in formulations of these **products** allowed the **parameters** which influence the efficiency of transfection in hematopoietic cells to be analyzed and the observations were used to increase the efficiency of the products and to decrease the toxicity of the vectors which could be good candidates for gene transfer protocol in hematology. (0 ref)

L4 ANSWER 15 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1994-07897 BIOTECHDS
TI Degradation of penicillin-V in fermentation media; unfavorable benzylpenicillin degradation during Penicillium chrysogenum fed-batch culture and the comparison of chemically defined and complex culture medium
AU Christensen L H; *Nielsen J; Villadsen J
CS Univ.Denmark-Tech.
LO Department of Biotechnology, Technical University of Denmark, DK-2800 Lyngby, Denmark.
SO Biotechnol.Bioeng.; (**1994**) 44, 2, 165-69
CODEN: BIBIAU
DT Journal
LA English
AB A reversed-phase liquid chromatography (LC) method that allowed for the simultaneous determination of side-chain precursors, hydroxylated benzylpenicillin (BP), penilloic-V acid, and 2 stereoisomers of penicilloic-V acid (PVA) was used to measure concentrations of BP and PVA during fed-batch fermentations of Penicillium chrysogenum. Model experiments were carried out with a chemically defined medium and a complex medium containing corn steep liquor. The degradation of BP was separated into a phosphate-catalyzed conversion of BP to PVA, overlaid by at least 1 other reaction in which BP was degraded to as yet unknown **products**. **Parameter** values for the phosphate-catalyzed degradation were independent of the type of fermentation medium. The rate of formation of other degradation products of BP was significantly higher in the complex fermentation medium with corn steep liquor than in the chemically defined medium. This may be a severe problem when carrying out repeated fed-batch fermentations where corn steep liquor, and consequently phosphate also, are added at times when the BP concentration is relatively high. (10 ref)

L4 ANSWER 16 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1994-06060 BIOTECHDS
TI Biotechnology product validation. Part 2: a logical plan; a review
AU Akers J; McEntire J; *Sofer G
CS Akers-Kennedy+Ass.; Appl.Anal.Ind.; Pharmacia-Biotech
LO Pharmacia, 800 Centennial Avenue, Piscataway, NJ 08854, USA.
SO Biopharm Manuf.; (**1994**) 7, 2, 54-56
CODEN: BPRME5
DT Journal
LA English
AB An overview is presented which discusses validation requirements during product development, pilot scale, and production. The following are covered: i. product development - **product** characterization, purification design **parameters**, cGMP issues; ii. pilot scale - product campaigns, product characterization, purification scale-up and process monitoring, validation studies, cGMP issues; and iii. production scale - product characterization, purification scale-up, validation studies and formal validation, GMP and documentation issues. (1 ref)

L4 ANSWER 17 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1995-06410 BIOTECHDS
TI Advantages and disadvantages of glucose limitation in perfused mammalian cell cultures: analysis of a large-scale, high-density myeloma cultivation; NSO high cell density perfusion cell culture in a 200 l sparged culture vessel and antibody production (conference abstract)
AU Konstantinov K B; Mered M; Golini F
CS Miles
LO R & D, Miles Biotechnology, 4th & Parker Streets, P.O. Box 1986, Berkeley, CA 94701, USA.

SO Cytotechnology; (**1994**) 14, Suppl.1, 5.12
CODEN: 3514D ISSN: 0920-9069
Animal Cell Technology, Developments Towards the 21st Century, Veldhoven, The Netherlands, 14-16 September, 1994.
DT Journal
LA English
AB Mammalian cell cultivation feeding strategies based on glucose limitation may have large-scale industrial application. Glucose limitation has advantages, including control of glucose metabolism, reduced lactic acid concentration, lower osmolarity, and simplified feeding technique. This strategy was applied to the industrial scale perfused cultivation of a NSO myeloma cell line for antibody production. Glucose-limited and non-limited experiments were conducted in a 200 l sparged culture vessel. The NSO cells were cultured for several mth at high density (20-60 x 10 power 6 cells/ml). Process variables representing cell physiology and process efficiency were analyzed in terms of statistics and dynamics. The effect of glucose limitation on the specific metabolic rates, metabolic rate ratios, cell viability, cell size and morphology, **product** glycosylation, and economic **parameters** was assessed. Some variables were favored while others were changed adversely and irreversibly, resulting in suboptimal process performance. Thus glucose-limited feeding has potential for industrial application, but should be used cautiously. (0 ref)

L4 ANSWER 18 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1993-11329 BIOTECHDS
TI Three different 2,3-dihydroxybiphenyl-1,2-dioxygenase genes in the Gr am-positive polychlorobiphenyl-degrading bacterium Rhodococcus glober ulus P6;
gene cloning, expression in Escherichia coli and role in polychlorina ted biphenyl degradation
AU Asturias J A; Timmis K N
LO Department of Microbiology, National Research Center for Biotechnolog y, D-3300 Braunschweig, Germany.
SO J.Bacteriol.; (**1993**) 175, 15, 4631-40
CODEN: JOBAAY
DT Journal
LA English
AB Rhodococcus globerulus P6 (Acinetobacter sp. P6, Arthrobacter sp. M5, Corynebacterium sp. MB1) degrades a wide range of polychlorinated bi phenyl (PCB) congeners. Clones contained in a cosmid pLAFR3 gene ban k of P6 were tested for 2,3-dihydroxy-1,2-dioxygenase (2,3-DHBD) acti vity by spraying with a solution containing 2,3-dihydroxybiphenyl. 1 3 Colonies which turned yellow were isolated. Examination of the res triction patterns of plasmids isolated from these clones revealed 3 r estriction groups: 2,3-DHBDI was encoded by the gene bphC1 of group I ; 2,3-DHBDII by gene bphC2 of group II; and 2,3-DHBDIII by gene bphC3 of group III. The bphC1 gene was expressed in Escherichia coli DH5- alpha from the lac promoter of plasmid pBluescript. The bphC2 and bp hC3 genes were expressed in E. coli independent of vector transcripti on signals. The 3 genes encoded narrow substrate-specificity enzymes that catalyzed meta-cleavage of the first aromatic ring. They showe d no hybridization homology to each other or to bphC genes in other b acteria, and their **products** had different kinetic **parameters** and sens itivities to inactivation by 3-chlorocatechol. (51 ref)

L4 ANSWER 19 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1993-08391 BIOTECHDS
TI Modeling of enzymatic reaction with emulsion liquid membrane;
mathematical model compared to experimental results obtained for L-amino acid production
AU Chang J H; *Lee W K
LO Department of Chemical Engineering, Korea Advanced Institute of Science and Technology, 373-1, Kusong Dong, Yusung Gu, Taejon, Korea.

SO Chem.Eng.Sci.; (**1993**) 48, 13, 2357-66
CODEN: CESCAC
DT Journal
LA English
AB A mathematical model was developed for analyzing the rate of reaction using an enzyme encapsulated by a water/oil emulsion. The proposed mechanism of the enzyme emulsion-liquid membrane model was based on the simple-diffusion (type 1) facilitation of the permeation of product. Input to the model was the phase and chemical equilibria at the interface of external-emulsion and internal-membrane pulse. The developed model considered the reactivity of internal enzyme, intra-emulsion diffusion and peri-emulsion mass transfer for both substrate and **product**. All **parameters** needed for modeling were estimated without the use of adjustable parameters, but only using the thermodynamic, transport, emulsion and enzyme properties. The proposed model predicted satisfactorily the experimental results of the enzymatic hydrolysis of an L-amino acid ester into an L-amino acid, an important step in the production of optically pure amino acids. (27 ref)

L4 ANSWER 20 OF 63 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1993-12525 BIOTECHDS
TI Monitoring the efficacy of bioremediation;
sampling, controlling and monitoring methods
AU Heitzer A; Sayler G S
LO Department of Microbiology, Center for Environmental Biotechnology, The University of Tennessee, 10515 Research Drive, Suite 100, Knoxville, TN 37932, USA.
SO Trends Biotechnol.; (**1993**) 11, 8, 334-43
CODEN: TRBIDM
DT Journal
LA English
AB The general acceptance of bioremediation technology as an environmentally sound and economic treatment for hazardous waste requires the demonstration of its efficacy, reliability and predictability, as well as its advantages over conventional treatments. An effective monitoring design includes protocols for treatment-specific, representative sampling, control and monitoring. These should take into account abiotic and biotic pollutant fate processes in all relevant process compartments. A number of well-established and novel chemical and molecular biological monitoring techniques and parameters is available. Logical and balanced combinations of chemical monitoring **parameters** (pollutant, intermediates, end **products**, terminal electron acceptors, toxicity assessment) and biological monitoring parameters (population size and diversity, specific activities) should be used to demonstrate complete degradation and detoxification of a hazardous waste as well as the biological nature of the process. At each process-scale level, a set of general criteria should be used for a systematic evaluation of overall efficacy. (37 ref)

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FILE 'BIOTECHDS' ENTERED AT 13:52:37 ON 13 MAY 2005
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L2 0 S L1 AND (WEIGHT (3A) PROPERTY)
L3 63 S L1 AND PY<2000
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         28323 PRODUCTS
         61034 PRODUCT
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          2333 PROPERTY
         18591 PROPERTIES
         20334 PROPERTY
                 (PROPERTY OR PROPERTIES)
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L6    ANSWER 1 OF 30  BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN    2005-09540  BIOTECHDS
TI    Kinetic model of the hydrolysis of polypeptides catalyzed by
      Alcalase((R)) immobilized on 10% glyoxyl-agarose;
         immobilized alcalase-catalyzed protein hydrolysis and kinetic model
         for kinetic study
AU    TARDIOLI PW; SOUSA RS; GIORDANO RC; GIORDANO RLC
CS    Univ Fed Sao Carlos
LO    Giordano RLC, Univ Fed Sao Carlos, Dept Engn Quim, Rodovia Washington
      Luiz,Km 235, BR-13565905 Sao Carlos, SP, Brazil
SO    ENZYME AND MICROBIAL TECHNOLOGY; (2005) 36, 4, 555-564    ISSN:
      0141-0229
DT    Journal
LA    English
AB    AUTHOR ABSTRACT - The sequential hydrolysis of cheese whey proteins can
      improve physical, chemical and organoleptic properties of this
      dairy by-product, increasing its applications in the food and
      pharmaceutical industry. The hydrolysis of polypeptides (50degreesC, pH
      9.5), catalyzed by Alcalase((R)) immobilized on 10% agarose (weight
      basis), activated with linear aliphatic aldehyde groups
      (glyoxyl-agarose), is studied here. The reaction substrate (polypeptides)
      is the product of a previous, sequential hydrolyses of cheese
      whey proteins by trypsin, chymotrypsin and carboxypeptidase A. A
      Michaelis-Menten model with product inhibition was fitted to
      the experimental data after long-term batch assays. Kinetic
      parameters k. K-M, and K-I Were Correlated with respect to the
      degree of hydrolysis of the substrate in the upstream proteolyses, thus
      providing a general, semi-empirical rate equation. With this approach,
      the kinetic model may be included in process optimization
      algorithms, which may span different regions of operation for the
      proteolytic reactors. Parameters k, K-M and K-I ranged from
      0.005 to 0.029 mmol/min/U-BAEE, from 4.0 to 13.7 mM. and from 0.19 to
      1.56 mM. respectively, when the previous degree of proteolysis
      (pre-hydrolysis) changed from 20 to 2%. (C) 2004 Elsevier Inc. All rights
      reserved. (10 pages)

L6    ANSWER 2 OF 30  BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN    2004-14637  BIOTECHDS
TI    Designing 3 dimensional (3D) protein model, by identifying common
      structural blocks (CSBs) among family members, aligning sequence,
      defining 3D structure of CSBs, global constraints, selecting rotamers,
      determining, optimizing 3D structures;
         protein 3D structure coordinate model and computer model for use in
         drug screening
```

AU ANDRE F; DELAFORGE M; LOISEAU N
PA COMMISSARIAT ENERGIE ATOMIQUE; CNRS CENT NAT RECH SCI
PI WO 2004038655 6 May 2004
AI WO 2003-IB5134 28 Oct 2003
PRAI US 2002-421569 28 Oct 2002; US 2002-421569 28 Oct 2002
DT Patent
LA English
OS WPI: 2004-389974 [36]
AB DERWENT ABSTRACT:

NOVELTY - Designing 3-dimensional- (3D) model of protein comprising identifying common structural blocks (CSBs) among members of the family, aligning primary amino acid sequence, aligning protein as compared on first alignment to obtain second alignment, defining 3D structure of CSBs of the protein, defining global constraints, selecting rotamers, determining family of 3D model structures of protein, **optimizing** models by dihedral angles, is new.

DETAILED DESCRIPTION - Designing (M1) a 3-dimensional- (3D) model of a protein, the 3-D representation of at least three family members has already been experimentally obtained, (the 3D representation presenting similarities), involves: (a) identifying common structural blocks (CSBs) among the members of the family; (b) aligning the amino-acids primary sequence of the family members according to the structural similarities, represented by the CSBs, in order to obtain a first alignment; (c) aligning the protein as compared on the compared on the first alignment, in order to obtain a second alignment, where: (a) alignment of the protein is performed in order to **optimize** the amino acids alignment between the protein and the first alignment, when one or more consensus amino-acid exists in the aligned CSBs in the first alignment, and in the amino-acid sequence of the protein, the consensus amino acids are anchors of the second alignmen; and (b) no insertion or deletion of amino acids can be performed in the aligned CSBs, where insertion or deletions are possible in out-of-block regions, if better to align the primary amino acids sequences; (d) defining the 3-D structure of CSBs of the protein, according to the 3-D structure of the CSBs of the family members; (e) defining of the global constraints (distance and angular constraints) derived from the comparisons of the structural templates in CSBs, and definition of the local constraints (distance and angular constraints) for the atoms of residues that are not structurally determined after step (d), (that are not in the CSBs); (f) selecting rotamers; (g) determining a family of 3-D model structures of the protein, taking into account the 3-D structure of CSBs obtained in step (d), the global and local constraints defined in step (e), and the rotamers defined in step (f); (h) **optimizing** the family of 3-D models obtained in step (g), by dihedral angles phi and psi of all residues located in CSBs are defined as constraints, given by the average values of corresponding phi and psi angles in the family members +/- the calculated standard deviation. INDEPENDENT CLAIMS are also included for: (1) a 3-D structure model of a protein (I), obtained by (M1); (2) designing (M2) a protein, biological functions of which are altered comprising obtaining a 3-D model of the protein by (M1), analyzing the obtained model and determining the amino acids that are putatively involved in the biological functions of the protein, changing the amino acids by mutating the corresponding nucleotides on the nucleic acid sequence coding for the protein, in order to obtain a mutated protein having altered **properties**; (3) a computer-assisted method (M3) for performing restrained dynamics docking of a substrate on an enzyme, a 3-D structure of which is available, comprising: (a) determining a force field, and independently simulating the presence of the enzyme in the force field; (b) minimizing the potential energy (Ep) linked to the force field of the 3-D structure, where the spatial position of some atoms of the enzyme is fixed, and where the other atoms are mobile, by allowing mobility of the mobile atoms, by simulating an increase in temperature (in order to give kinetic energy) and minimizing the potential energy by Rex-specifying the temperature as 0 kelvin (K); (c) optionally repeating

step (b) in order to obtain other Ep minima, where the Ep minima are such that the structure of the protein remains folded; (d) minimizing Ep in the force field of the 3-D structure, where all the atoms of the protein are mobile, by simulating an increase in temperature (in order to give kinetic energy), and minimizing the potential energy by Rex-specifying the temperature as 0 kelvin (K); (e) simulating, at 0 K the presence of the substrate next to the enzyme; (f) optionally generating a molecular dynamics simulation on the substrate and enzyme (simulating an increase in temperature, in order to allow mobility of the atoms); (g) generating some constraints to the substrate, in order to impose that it has interaction with the enzyme; (h) generating a molecular dynamics simulation on the substrate and enzyme, with the constraints imposed in step (g); (i) optionally, generating a molecular dynamics simulation on the substrate and enzyme without the constrains of step (g); and (4) a computer-assisted method (M4) for performing restrained dynamics docking of at least two substrates on an enzyme, a 3-D structure of which is available involves performing the step of (M3) with a first substrate and repeating the steps with a second substrate when the first substrate reaches an unconstrained state after molecular dynamics simulation.

BIOTECHNOLOGY - Preferred Method: In (M1), the rotamers in step (f) are selected from the couples according to the tables of Dunbrack and karplus, where the choice of rotamers of a given amino acid is guided by the most favored zones in Ramachandran chi1, chi2 maps. The step (g) is performed with the DYANA software. The **optimization** step (h) involves the use of the X-Plor software, as described in A.T.Brunger, X-PLOR, version 3.1. The protein is a cytochrome P450 subfamily 3A comprising mammal and human cytochromes P450 3A. The mammal cytochrome P450 3A is chosen from CYP3A6 (having a fully defined sequence of 501 amino acids as given in the specification), CYP3A12 (having a fully defined sequence of 503 amino acids as given in the specification), CYP3A29 (having a fully defined sequence of 503 amino acids as given in the specification), and CYP3A13 (having a fully defined sequence of 503 amino acids as given in the specification). The human cytochrome P450 subfamily 3A is chosen from CYP3A4 (having a fully defined sequence of 502 amino acids as given in the specification), CYP3A7 (having a fully defined sequence of 503 amino acids as given in the specification), CYP3A5 (having a fully defined sequence of 502 amino acids as given in the specification) and CYP3A43 (having a fully defined sequence of 503 amino acids as given in the specification). The family members that are used for performing the first alignment for designing a 3-D model of CYP3A4 are chosen from Nor (having a fully defined sequence of 403 amino acids as given in the specification), Ery F (having a fully defined sequence of 403 amino acids as given in the specification), terp (having a fully defined sequence of 412 amino acids as given in the specification), Cam (having a fully defined sequence of 414 amino acids as given in the specification), BM3 (having a fully defined sequence of 471 amino acids as given in the specification) and 2C5 (having a fully defined sequence of 473 amino acids as given in the specification). The family members are preferably chosen from Ery F, BM3, 2C5 and CYP51 (having a fully defined sequence of 455 amino acids as given in the specification). In (M3), the fixed atoms in step (b) are the backbone atoms N-Calpha-CO in the first minimization step and only Calpha in subsequent minimization steps. The kinetic energy is simulated by temperature increase to about 100 K for about 5-20 ns. The force field in step (a) comprises forces linked to the distance between atoms, the angles of valence, the dihedral-angles, the deformation with regard to planar geometry, the electrostatic field, the Van der Waals forces or the hydrogen bonds. The constraints in step (g) are attraction constraints to force the substrate in the active site, and where the constraints are not prejudiced to the exact spatial conformation of the substrate in the active site. The constraints are final distance constraints between some atoms of the substrate and some atoms of amino-acids present in the active site. The step (f) and (h) is performed with a simulated temperature of between about 15 and 50 K. The step (i) is performed with

a simulated temperature of between about 200 and 350 K. The enzyme is a cytochrome P450 3A4, comprising mammal and human cytochromes and the structure is the structure obtained by (M1). The substrate is a small organic compound which size can range for example from molecular weight (MW) 288 (testosterone) to MW 1203 (cyclosporine A). The substrate is preferably testosterone. In (M4), the first and second substrates are the same or different molecule. The first and second substrates display an allosteric or synergistic effect. At least one of the substrates is an inhibitor or displays an inhibitor-based mechanism. At least one of the substrates in an agonist. (M4) involves successively repeating the steps with third, fourth, or fifth substrate, some of them being the same or different molecules. Preferred Model: In (I), the protein is a cytochrome P450 3A4 or 3A7. The residues Cys97, Arg104, Phe101, Phe107, Phe247, Phe303 and Cys376 are involved in the CYP 3A4 for the recognition and uptake of the substrate at the entry site, and its binding into the active site. The residues Gln79, Phe102, Arg105, Arg106, Phe108, Phe248, Phe304 and Glu374 are involved in the CYP 3A7 for the recognition and uptake of the substrate at the entry site, and its binding into the active site. (I) has the 3-D atomic coordinates as provided in the patent specification (atomic coordinates of structural models obtained by applying DYANA calculation to target protein sequences CYP3A4 and CYP3A7).

USE - (M1) is useful for designing a 3-dimensional- (3-D) model of a protein, the 3-D representation of at least three family members has already been experimentally obtained, (the 3-D representation presenting similarities). (M3) and (M4) are useful for performing restrained dynamics docking of a substrate on an enzyme. (M3) or (M4) is useful for screening, designing or identifying natural, unnatural substrates or substrate analogs, as well as inhibitors, activators or modulators of the enzyme. (M3) or (M4) is useful for determining the effect of a first substrate on a second substrate and can be applied to pharmaceutical **products**. (M3) or (M4) is useful for determining the effect of the first testosterone molecules on a second testosterone molecule or on a alpha-napthoflavone molecule. (M3) or (M4) is useful for determining the oxidative modification of the substrate according to the proximity to the heme of a part of the substrate, for performing dynamic docking of the metabolite either in the absence or in the presence of the second substrate in the computed simulation and to compare the energy of the bound metabolite relatively to the energy of its parent substrate bound, in order to determine if the exit of the given metabolite from the enzyme is favored or not (all claimed).

EXAMPLE - Determination of 3D structure of cytochrome P450 3A4 (CYP450 3A4) was as follows. The coordinates of the six P450 crystal structures: P450cam (3cpp), P450TERP (1cpt), P450BM-3 (2 hpd), P450eryF (loxa), P450 nor (1 rom) and P450 2c5 (1 dt6) were retrieved from the Brookhaven protein data bank. The first key point of this homology modeling study was the identification of the structural elements (designated as CSBs for common structural blocks) conserved among the family of cytochromes P450 of known 3D structures, and the localization of these elements in the target sequence. These two tasks were performed using the GOK software and were well described in a article Minoletti et al., Proteins, Structure, Function and Genetics, 2002. The multiple sequence alignment derived from the CSB identification was then used to build a similarity profile. The similarity score was calculated using BLOSSUM62 matrix. Once the alignment was completed, the 3D model rebuilding process can incorporate the atom Cartesian coordinates of the template structures only for amino acids located in structurally conserved regions (the CSBs). The coordinates of any of the template structures can be used for determining the final template. In each CSB, amino acid positions have been renumbered according to the sequence of human P450 3A4. At a given position, when residues were identical between all the template structures and the target sequence, the 3D coordinates of the reference residues were purely assigned to the modeled (target) residue. When residues differ, only the coordinates of the backbone atoms

were assigned (Calpha), the sometimes Cbeta when they exist. Side chains were rebuild from libraries giving the most probable rotamers for each amino acid. In some cases, it was possible to superimpose the positions of carbon atoms of lateral chains up to ranks gamma and delta along the side chain, thus explicity defining a unique rotamer. Constraints derivation and rebuilding was performed as follows. The number of constraints corresponding to all atom-atom distances, for example, would be prohibitive for a protein of the size of the P450. Previous nuclear magnetic resonance (NMR) studies have shown that local constraints were sufficient to allow a correct reconstruction of a structure. This drastically reduced the number of constraints needed, and increased the flexibility of the model. In addition, similarly to what is done in protein structure determination by NMR, we can build a family of structures instead of a single model. This allows an easier analysis of the well or less well-predicted regions. This was also an advantage for the analysis of the side-chain positions, particularly in prevision of a substrate docking study. Finally, the loops were passively reconstructed with the rest of the structure. The only specific information introduced in variable regions was to guide all their residues to an allowed region of the Ramachandran diagram. Indeed, analysis of well-defined structures showed that nearly all residues, including those of the loops, should belong to an allowed region. The lower the proportion of residues found outside the allowed Ramachandran regions, the better the structure was. In proteins, the preferential orientation of the side chain (60degrees,-60degrees, 180degrees) depended on the local conformation of the residue, and thus on the nature of the secondary structure in which the residue in involved. According to the rotamer library built by Karplus and coll, to a given (phi, psi) couple in the Ramachandran diagram can be associated a specific rotamer for each type of residue. These tables have been used to determine the most probable rotamer for each residue located in CSB, except when there were conserved atoms in the side chain that assign unambiguously a rotamer (chi1, chi2). The selected (chi1, chi2) couples were included in the above-mentioned set of angle 761 dihedral constraints. Structure calculation and **optimization** were performed using the DYANA software and the 58506 distance and 761 angular constraints. Families of structures were generated. The energy of each structure was minimized with the procedure vtfmin in DYANA. The others were further **optimized** by using the X-PLOR software. A set of constraints was added at this stage in order to guide the loop residues to the nearest allowed region in the Ramachandran diagram. The topology and **parameter** files of CHARMM22 were used. The electrostatic term was turned off. The DYANA software was unable to deal with disconnected objects. A new residue type was, thus, added to the standard amino acid library to take into account the presence of the heme. This residue was obtained by combining the heme to a cysteine and was inserted at position 441 in the sequence of the protein. The model of the protein depleted of its first 50 residues (N-terminal domain) was rebuilt. This segment was highly hydrophobic, and supposed to form the anchor of the protein in the membrane. There was no structural information about this putative transmembrane domain, and this segment was thus not incorporated into the modeling process, and in the final model. Such a free segment (with no constraints) would perturbate the convergence of computation or the stability of the whole rebuilt structure. The quality of the various structures **optimized** under XPLOR was checked for the stereochemical quality (backbone and side chain conformation) by PROCHECK. The Ramachandran plot showed that our six-template approach generated converging models, possessing the same fold. The lowest energy models had 73% of their non-glycine and non-proline residues with phi-psi conformation in the most favored regions of the Ramachandran plot (core region), 20% in additional allowed regions, and 5% in the generously allowed regions. Only 2.3% (9 residues) had their (phi-psi) conformation in disallowed regions. When compared to the CYP2C5 crystal structure, it can be noticed that the CYP3A4 model exhibits a good 3D similarity in the global fold than expected, since

this structure counts only for one in the six-template approach. The substrate recognition sites (SRS) were associated with the active site and were located in the less conserved regions of the CYPs, thus possibly accounting for the various substrate specificity among P450s. When comparing out various **optimized** structures, it was found that SRS1 (100-125 includes helix B), SRS 2(205-218, includes C-terminus of helix F), and SRS3 (237-249, includes N-terminus part of helix G) were located in less-defined regions, with significant variability in spatial position (flexibility). These regions correspond also to parts of the sequence that were less well-aligned. At the opposite, the SRS4 (295-320, central part of helix I), SRS5 (363-380, C-term of helix K and beta-sheet beta1-4) and SRS6 (470-490, beta-sheets beta4-1 and beta4-2) were well-defined fragments of the structures. SRS4 and SRS5 segments in particular were correlated to regions in the sequence that are unequivocally aligned. The only model structure of CYP3A4 that has been described in the literature and that we can handle for structural comparison, is that of Szklarz and Halpert, derived from a multiple-template approach (four-bacterial template) (Szklarz and Halpert 1997). Roughly, the same secondary structures were identified, but divergences were found in SRS location between their model and those derived from the present approach. SRS4 and SRS5 matched well, but SRS2 was shifted (divergence in the position of helix F along the sequence), while SRS1 (helix B'), SRS3 (helix G) and SRS6 (sheet beta4) were more notably displaced. (193 pages)

L6 ANSWER 3 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 2004-16115 BIOTECHDS
TI Determining pyrophosphate, useful e.g. for **optimizing** polymerase chain reaction conditions or nucleic acid synthesis, uses polymerase with pyrophosphorolysis activity and oligonucleotide as indicator;
thermophilic DNA-polymerase and thermophilic RNA-polymerase for DNA amplification and diagnosis
AU PETERS L
PA EPPENDORF AG
PI DE 10250491 13 May 2004
AI DE 2002-1050491 29 Oct 2002
PRAI DE 2002-1050491 29 Oct 2002; DE 2002-1050491 29 Oct 2002
DT Patent
LA German
OS WPI: 2004-392632 [37]
AB DERWENT ABSTRACT:
NOVELTY - Determining pyrophosphate (PP) in reaction mixture and biological samples comprising using at least one polymerase (I) with pyrophosphorolysis activity and an indicator substrate (I) that generates a signal depending on the amount of PP present, is new.

DETAILED DESCRIPTION - Determining pyrophosphate (PP) in reaction mixture and biological samples using at least one polymerase (I) with pyrophosphorolysis activity and an indicator substrate (I) that generates a signal depending on the amount of PP present. The new feature is that (I) is an at least partly double-stranded (ds) oligonucleotide (ON) of random sequence which is labeled at the 3'-end with a detectable functional group or molecule (A). In presence of (I), the labeled 3'-terminal nucleotide (nt), with attached (A), is released with consumption of a PP molecule, and this release is quantified to indicate the PP content. An INDEPENDENT CLAIM is also included for a reagent kit for **optimization** of PCR or other in vitro DNA/RNA synthesis reactions containing (I); polymerase; nucleotides; control template(s); instructions and **optimized** examples.

BIOTECHNOLOGY - Preferred Materials: (A) is a radioisotope, e.g. tritium; a ligand, e.g. biotin; a low molecular weight hapten, e.g. fluorescein; a group detectable by mass spectrometry or an electroluminescent complex; or (I) is labeled with a fluorophore/quencher pair, one component of which is displaced by polymerase reaction.

Preferably the phosphodiester bond in the 5'-direction of the labeled nt is modified so that further pyrophosphorolytic degradation is inhibited, e.g. as a phosphorothioate. (I) is particularly a ds ON with an overhang at at least one 5'-end; the ds region contains 10-30 bp and the overhang(s) 1-10 bases. Particularly is has a hairpin structure and may be a DNA/RNA hybrid with at least two ribonucleotide monophosphate residues on at least one 3'-end, and then the 3'-terminal resiude is labeled with quencher or fluorophore. The reverse reaction, i.e. pyrophosphorolytic cleavage of the signal-generating 3'-nt is inhibited by adding at least one unmodified nt that has the same base-pairing **properties** as the 3'-terminal base but higher binding affinity for (I), and present at above the concentration of (I). Alternatively, the base opposite the 3'-end in the antisense strand is a universal base (e.g. deoxyinosine) that forms equally weak bonds with all four natural deoxynucleotide triphosphates. Also the (I)-induced incorporation of nt at the 3'-end is blocked or reduced e.g. by using a 2',3'-dideoxy 3'-terminal residue, by esterifying the 3'-hydroxy in this residue with an alkyl spacer, or by having the 5'-overhang made at least partly from synthetic universal bases. The base sequence of (I) is chosen so that is only very rarely encountered in the genome being examined, particularly it comprises many linear repeats of CA or GT. Suitable enzymes with both polymerase and pyrophosphorolytic activity include thermophilic DNA polymerases without 3'-5' (proofreading) activity, e.g. Taq. Alternatively, a thermophilic RNA polymerase is used, exclusively for detection of (I), while a DNA polymerase provides the PP required for the process. Preferred Process: The labeled 3'-terminal nt released is measured in a separate detection reaction or during the decomposition reaction. The method is applied to e.g. blood, serum, urine, cerebrospinal or synovial fluids, where the PP concentration may be indicative of disease. Endogenous pyrophosphorylation inhibitors are removed in a preliminary step and pyrophosphorylation of (I) is coupled, in the same reaction vessel, with a pyrophosphate-releasing reaction. Particularly PP is detected by an isothermal DNA/RNA polymerase reaction and (I) does not function as a false primer during the amplification.

USE - The method is particularly useful for real-time quantification of PCR **products** (e.g. in body fluids where the level of PP may indicate disease) or for RNA synthesis/polymerization processes in in vitro or in vivo transcription systems; for determining tissue-specific promoter activity; for detecting tissue-specific transcription factors (inhibitors, effectors or enhancers); for **optimization** of PCR or other nucleic acid synthesis reactions; and for **optimization** or validation of programmable thermostats or their reaction **parameters**.

ADVANTAGE - The method provides simple and very specific detection of PP, even at very low concentrations. (10 pages)

L6 ANSWER 4 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 2004-18718 BIOTECHDS
TI Method for recombination of double-stranded DNA from different diversification strategies, useful e.g. for assembling synthetic genes, based on amplification with primers that create strand breaks; DNA amplification and computer model for use in DNA recombination
AU BERNAUER H S; MUELLER K
PA ATG BIOSYNTHETICS GMBH
PI DE 10234577 17 Jun 2004
AI DE 2002-1034577 30 Jul 2002
PRAI DE 2002-1034577 30 Jul 2002; DE 2002-1034577 30 Jul 2002
DT Patent
LA German
OS WPI: 2004-488999 [47]
AB DERWENT ABSTRACT:
NOVELTY - Achieving free combinability of different double-stranded (ds) DNA molecules (A) from different diversification strategies, in a standardized process comprises: (1) preparing at least two different (A);

(2) amplifying (A) by a molecular biology method, using oligonucleotide primers that can cause cleavage of a DNA strand; (3) optionally fragmenting the amplification **products** to subfragments; (4) generating compatible, complementary ends that allow a combinatorial mixing of sequences; and (5) sequence-specific combination of at least terminally homologous DNA fragments from different diversification strategies.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) kit comprising components for performing the new method and instructions; and (2) computer program for (i) determining all **parameters** of a diversification strategy, from different partial strategies, (ii) for calculation and (iii) for making suggestions for planning and performing experiments.

BIOTECHNOLOGY - Preferred Process: This is applied to at least two (A) with at least partial homology at their termini, and during polymerization one or more nucleotide analogs (X) is/are incorporated, either at targeted positions or randomly, especially incorporation of deoxyuracil (dU). Particularly this is achieved by using a dU-containing primer during strand amplification of a template and/or by including dU triphosphate in the polymerization reaction mixture. Some (X) can then be removed in targeted manner, particularly by using dU-glycosylase in a cleavage reaction. Optionally an affinity marker (AM), e.g. a reactive biotin derivative, is attached to the terminus, or to a functional group, on at least one cleavage site, before or after cleavage. At least part of one of the two single strands on one or both sides of the ds molecule, are separated using AM, or the cleaved DNA fragment is 'titrated out' using an excess of a complementary molecule. Preferably from a mixture of at least 2 DNA fragments those with overlapping single-stranded ends are generated and are able to anneal in all possible combinations. A set of many amplification primers is used that (i) contain at least one (X); (ii) can generate homologies or deletions; (iii) comprise complementary, terminal sequences of different amplification **products**; (iv) are derived from a sequence in internally identical or partially randomized manner; (v) are fully randomized or (vi) are overlapping or non-overlapping. Both the position of the self-break points and the recombination frequency of individual strands are random, or are calculated from an algorithm that identifies the oligonucleotides to be used on the basis of amplicon sequence, so as to define ideal break points. The process is used to generate sequence diversity, including preparation of heteroduplex DNA, e.g. chimeras and coding sequences. It can be fully automated and/or miniaturized (in microfluidics systems), and may include further mixing and recombination. Any sequence gaps or nicks can be repaired and/or ligated before ligation and incorporation into a cell system. Cell lines containing (re)combined DNA are isolated, replicated, than analyzed by bioassay for measurement and selection of desired **properties**. The entire process can be repeated for evolutive/selective improvement of **properties**, or additional iterative **optimization** is done.

USE - The method is used to assemble synthetic genes; for in vitro DNA splicing; for recombination of partially homologous DNA molecules for variation or **optimization** of functional or other **properties**; to prepare chimeric genes, chromosomes, vectors etc.; to construct vectors from any selected components and to make chimeric fusion genes.

ADVANTAGE - The method is based on a very reproducible incorporation of deoxynucleotide phosphates, at various concentrations, so makes possible cleavage of the phosphodiester backbone at one or more predetermined positions. This allows recombination of DNA molecules from different diversification strategies at the fragment level. (18 pages)

L6 ANSWER 5 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 2003-26379 BIOTECHDS
TI New nucleic acid molecule termed mel genes encoding polypeptide synthase and non-ribosomal peptide synthetase that are involved in synthesis of

melithiazol displaying enhanced fungicidal activity; recombinant polypeptide-synthase, non-ribosomal peptide-synthetase and phosphopantetheinyl-transferase production by vector-mediated mel gene transfer and expression in myxobacterium, Pseudomonas, actinomycete and Escherichia coli host cell for fungicide production

AU MUELLER R; WEINIG S; BLOECKER H; HOEFLE G; SASSE F

PA GBF GES BIOTECH FORSCHUNG GMBH

PI WO 2003080828 2 Oct 2003

AI WO 2003-EP2987 21 Mar 2003

PRAI EP 2002-6389 21 Mar 2002; EP 2002-6389 21 Mar 2002

DT Patent

LA English

OS WPI: 2003-767886 [72]

AB DERWENT ABSTRACT:

NOVELTY - Nucleic acid molecule (I) encoding a polypeptide involved in the synthesis of melithiazol (II), nucleic acid chosen from nucleic acid encoding polypeptide Mel B-H, open reading frame (ORF) 1-7, nucleic acid molecules termed mel B-H, ORF (I)-(VII), and a nucleic acid molecule encoding a (poly)peptide being involved in synthesis of (II), is new.

DETAILED DESCRIPTION - Nucleic acid molecule (I) encoding polypeptide involved in the synthesis of melithiazol (II), the nucleic acid is chosen from: (a) a nucleic acid encoding the polypeptide having fully defined sequence of 1050 (Mel B), 1323 (Mel-C), 3284 (Mel-D), 1912 (Mel-E), 1356 (Mel-F), 1747 (Mel-G), 329 (Mel-H), 232 (ORF-1), 452 (ORF-2), 320 (ORF-3), 118 (ORF-4), 335 (ORF-5), 301 (ORF-6), 595 (ORF-7) amino acids, as given in the specification; (b) nucleic acid molecules having fully defined DNA sequence of 3153 (mel B), 3972 (mel C), 9855 (mel D), 5739 (mel E), 4071 (mel F), 5244 (mel G), 990 (mel H), 699 (S1), 1359 (S2), 963 (S3), 357 (S4), 1008 (S5), 906 (S6), 1788 (S7) nucleotides, as given in the specification; (c) a nucleic acid molecule encoding a (poly)peptide being involved in synthesis of (II) and having an amino acid sequence that is at least 80%, preferably at least 90% and most preferably at least 99% identical to Mel B-H, ORF 1-7 or amino acid sequence encoded by mel B-H, S1-S7; (d) a nucleic acid molecule encoding a (poly)peptide being involved in synthesis of (II) and having an amino acid sequence of Mel B-H, ORF 1-7 or amino acid sequence encoded by mel B-H, S1-S7 with at least one conservative amino acid substitution, or having an amino acid sequence that is an isoform of the amino acid sequence of Mel B-H, ORF 1-7 or amino acid sequence encoded by mel B-H, S1-S7; and (e) a nucleic acid molecule encoding a (poly)peptide being involved in the synthesis of (II) the complementary strand of which hybridizes under stringent conditions to the DNA molecule encoding the amino acid sequence described above. INDEPENDENT CLAIMS are also included for the following: (1) a gene cluster (III) comprising at least two (I); (2) a vector (V1) comprising (I); (3) a vector (V2) comprising several different (I); (4) a vector (V3) comprising (III); (5) a host cell (IV) transformed or transfected with any one of (V1)-(V3); (6) a host cell (V) transformed with several (V1); (7) a polypeptide encoded (I) or produced by using (IV) or (V); (8) improving (M1) a pharmacological **property** of (II) comprises: (a) replacing in a host cell transformed or transfected with (V2) or (V3), or in (IV), at least one (I) by corresponding nucleic acid molecule from a heterologous source, and testing for the desired improvement of the pharmacological **property**; (b) subjecting, in a host cell transformed or transfected with (V1) or (V2), (I) to mutagenesis, and testing for the desired improvement of the (poly)peptide encoded by the nucleic acid molecule, combining the nucleic acid molecule in expressible from in a host cell with the remainder of the nucleic acid molecule present (II) or with corresponding nucleic acid molecules from a heterologous source in expressible form, allowing production of (II), and testing for the desired improvement of the pharmacological **property**; and (c) deleting from or inactivating in a host cell transformed or transfected with (V3) one or more (I), or providing a host cell transformed or transfected with (V2) or with several of vectors comprising each

different (I), where several of different (I) is less than the genes contained in (III) and where the host cell further comprises a nucleic acid molecule encoding a phosphopantetheinyl transferase in expressible form, allowing for expression of the nucleic acid molecules, assessing whether a compound with fungicidal activity is produced and/or assessing whether the compound produced has an improved pharmacological **property**; (9) improving (M2) a desired **property** of or conferring a desired **property** to a secondary metabolite different from (II) comprises providing a host cell comprising a gene cluster or a part of a gene cluster, where the gene **products** of the gene cluster are involved and sufficient for the synthesis of the secondary metabolite, transforming or transfecting the host cell with at least one (V1) or (V2) or with several of vectors comprising each different (I), where several of different (I) is less than the genes contained in the (III), allowing for expression of the nucleic acid molecule, assessing whether a secondary metabolite is produced, and/or assessing whether the secondary metabolite produced displays a desired **property** or the improved desired **property**; (10) a melithiazol obtainable by (M1) or (M2); and (11) a composition comprising (II).

BIOTECHNOLOGY - Preferred Nucleic Acid: (I) is DNA or RNA. Preferred Host Cell: (IV) or (V) is a bacterium which is a myxobacterium, an actinomycete, a Pseudomonad or Escherichia coli. (IV) or (V) further comprises a nucleic acid molecule encoding a phosphopantetheinyl transferase in expressible form. Preferred Method: In (M1) (c), the deletion or absence of the nucleic acid molecule results in the absence of the first thiazol ring of (II). In (M1) or (M2), the pharmacological **property** is chosen from improved range of fungicidal activity, and lower toxicity for the human or animal patient. (M1) or (M2) further involves modifying (II) as a lead compound to achieve modified site of action, spectrum of activity, organ specificity, and/or improved potency and/or decreased toxicity (improved therapeutic index), and/or decreased side effects and/or modified onset of therapeutic action, duration of effect, and/or modified pharmakinetic **parameters** (resorption, distribution, metabolism and excretion), and/or modified physico-chemical **parameters** (solubility, hygroscopicity, color, taste, odor, stability, state) and/or improved general specificity, organ/tissue specificity and/or **optimized** application form and route. The modification comprises: (1) esterification of carboxyl groups (2) esterification of hydroxyl groups with carbon acids; (3) esterification of hydroxyl groups to phosphates, pyrophosphates or sulfates or hemi sulfates; (4) formation of pharmaceutically acceptable salts or complexes; (5) synthesis of polymers; (6) introduction of hydrophilic moieties, (7) introduction/exchange of substituents on aromates or side chains, or change of substituent pattern; (8) modification by introduction of isosteric or bioisosteric moieties; (9) synthesis of homologous compounds; (10) introduction of branched side chains; (11) conversion of alkyl substituents to cyclic analogues; or derivatization of hydroxyl group to ketales and acetales (sic); (12) N-acetylation to amides, phenylcarbamates; (13) synthesis of Mannich bases and imines; and/or (14) transformation of ketones or aldehydes to Schiff's bases, oximes, acetales, ketales, enolesters, oxazolidines, and thiazolidines.

ACTIVITY - Fungicide. No biological data is given.

MECHANISM OF ACTION - None given.

USE - (IV) or (V) is useful for producing (II) which involves culturing (IV) or (V) under suitable conditions and isolating (II) produced (IV) or (V) is useful for producing a (poly)peptide involved in the synthesis of (II) which involves culturing (IV) or (V) under suitable conditions and isolating the polypeptide produced. (M1) or (M2) is useful for producing a composition which involves performing (M1) or (M2) and further formulating (II) or the secondary metabolite thus obtained with a carrier or diluent (claimed). (I) encodes polypeptide involved in synthesis of (II) displaying enhanced fungicidal activity.

ADMINISTRATION - The composition is administered by intravenous,

intraperitoneal, subcutaneous, intramuscular, topical, intradermal, intranasal or intrabronchial route in dosages ranging from 0.001-1000 microg.

EXAMPLE - Melittangium lichenicola Me I 46, the organism which produced melithiazol, was cultivated in M7 medium (0.5% Probion ME 069, 0.1% CaCl2 H2O, 0.1, Mg2SO4 7H2O, 0.1% yeast extract, 0.5% soluble starch, 1% N-(2-hydroxyethyl)piperazine-N'-ethanesulfonic acid (HEPES), pH adjusted to 7.4 with 20% KOH, supplemented with 0.2% glucose and 0.1 mg/ml cyanocobalmin after sterilization). 100-ml batch cultures in 250 ml Erlenmeyer flasks were incubated at 30 degrees C on a gyratory shaker at 160 rpm for about 3 days. After harvesting the cells chromosomal DNA of M.lichenicola Me I 46 was isolated and used for the generation of a cosmid library. The resulting cosmid library consisted of 1920 clones, which were screened for the presence of melithiazol biosynthetic genes using fragments of genes mtaEFG of the myxothiazol biosynthetic gene cluster from Stigmatella aurantiaca DW4/3-1. The probes were derived from the regions coding for the putative O-methyltransferases of MtaE and MtaF, respectively the adenylation domain of MtaG. For the amplification of the probes the following primers were used. Primer METE 1 5' - cagagctcgaggtcatgttgcagtcgc-3', Primer METE 4 5' - gctctagatgagcccgaagcgcttggac-3', Primer FSW1 5' -aggtggggccgaagccgacgttg-3', etc. A standard polymerase chain reaction (PCR) was carried out using Taq polymerase. Cosmids M1 and M2 gave signals with the mtaE and mtaF derived probes, whereas cosmid M2 additionally hybridized with the mtaG derived probe. Analysis of the nucleotide sequence determined from the two cosmids revealed an overlap of 5372 base pairs, starting at position 34039. Comparison with the myxothiazol gene cluster showed colinearity of the melithiazol biosynthetic genes within the melC-melG region as judged by the structural similarities between the two molecules. (183 pages)

L6 ANSWER 6 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 2003-27053 BIOTECHDS
TI Determining fitness of potential crossover points on reference peptide sequence for **optimizing** directed points for directed mutation by calculating an overall value of a fitness **parameter** for chimeras with potential crossover point;
potential crossover point and mutation determination using computer bioinformatic software and hardware
AU GOVINDARAJAN S; GUSTAFSSON C; MINSHULL J S
PA MAXYGEN INC; MUNDORFF E C
PI WO 2003078583 25 Sep 2003
AI WO 2003-US7610 10 Mar 2003
PRAI US 2002-373591 18 Apr 2002; US 2002-363505 9 Mar 2002
DT Patent
LA English
OS WPI: 2003-779125 [73]
AB DERWENT ABSTRACT:
NOVELTY - Determining the fitness of multiple potential crossover points on a reference peptide sequence comprising, for each of the multiple potential crossover points on the reference peptide sequence, calculating an overall value of a fitness **parameter** from a multiple individual values of the fitness **parameter** for multiple chimeras having the potential crossover point under consideration, is new.

DETAILED DESCRIPTION - Determining the fitness of multiple potential crossover points on a reference peptide sequence comprising, for each of the multiple potential crossover points on the reference peptide sequence, calculating an overall value of a fitness **parameter** from a multiple individual values of the fitness **parameter** for multiple chimeras having the potential crossover point under consideration, is new. The method comprises: (a) for each of the multiple potential crossover points on the reference peptide sequence, calculating an overall value of a fitness **parameter** from a multiple individual values of the fitness **parameter** for multiple

chimeras having the potential crossover point under consideration; and (b) based on the respective overall values of the fitness **parameter** for the potential crossover points, choosing an actual crossover point for a chimeric peptide comprising a partial sequence of the reference peptide sequence. INDEPENDENT CLAIMS are included for the following: (1) a computer program **product** comprising a machine-readable medium on which is provided program instructions for determining the fitness of multiple potential crossover points on a reference peptide sequence, the program instructions comprising: (a) code for calculating, for each of the multiple potential crossover points on the reference peptide sequence, an overall value of a fitness **parameter** from multiple individual values of the fitness **parameter** for multiple chimeras having the potential crossover point under consideration; and (b) code for choosing, based on the respective overall values of the fitness **parameter** for the potential crossover points, an actual crossover point for a chimeric peptide comprising a partial sequence of the reference peptide sequence; (2) a computer-implemented method for determining the fitness of two or more potential crossover points; (3) selecting crossover points between two or more biomolecules; (4) a computer-readable medium comprising computer code that: (a) inputs a reference sequence of a reference biomolecule; (b) generates a contact map for the reference sequence; (c) aligns a first sequence and a second sequence with the reference replacing a subsequence comprising first and second crossover sites on the first sequence with a corresponding subsequence from the second sequences to produce a chimeric sequence; (d) comprises the chimeric sequence with the contact map to select two or more elements in the chimeric amino acid sequence that correspond to proximal elements in the contact map; and scores the selected elements; and (5) an integrated system for assessing crossover sites comprising the computer readable medium, and a graphical interface.

BIOTECHNOLOGY - Preferred Method: In determining the fitness of multiple potential crossover points on a reference peptide sequence, the crossover point is an intermediate point on the reference peptide sequence and the chimeric peptide comprises a partial sequence of the reference sequence terminating at the crossover point. The method further comprises producing at least one chimeric nucleic acid encoding the chimeric peptide. Producing at least one chimeric nucleic acid comprises recombining oligonucleotides, including at least one oligonucleotide encoding the chosen crossover point. The at least one oligonucleotide encoding the chosen crossover point comprises two partial sequences, one encoding a partial sequence of one parent peptide and another encoding a partial sequence of another parent peptide, with the two partial sequences meeting at a location in the oligonucleotide corresponding to the chosen crossover point. One of the parent peptides has a sequence comprising the reference peptide sequence. The other parent peptide is encoded by a nucleic acid from a gene family that includes a gene encoding the reference peptide sequence. The fitness **parameter** comprises a measure of a chimeric allele's ability to increase or decrease the binding specificity of a peptide. The fitness **parameter** comprises a measure of a chimeric allele's ability to preserve or improve the folding of the polypeptide. The method further comprises calculating an individual value of the fitness **parameter** for a chimera sequence having the potential crossover point under consideration. Calculating the individual value of the fitness **parameter** comprises: (a) aligning the chimera sequence to the reference peptide sequence; (b) identifying contacting residues of the chimera from a contact map; and (c) summing residue-residue potentials for contacting residues of the chimera. The contact map is a three-dimensional arrangement of residues in the reference peptide. The reference peptide sequence is the sequence of a naturally occurring peptide. The reference peptide sequence is the sequence of a non-natural peptide identified by a recombination or mutagenesis procedure. The choosing step comprises choosing multiple crossover points for multiple

chimeric peptides comprising partial sequences of the reference peptide sequence. The method further comprises producing a library of peptides comprising the multiple chimeric peptides. The one or more peptides of the library are produced by a method comprising expressing the one or more of peptides. The method further comprises: (a) providing an expression system from which a selected member of the library of peptides can be expressed; (b) cloning a polynucleotide encoding the selected member of the library of peptides into the expression system; and (c) expressing the selected member of the library of peptides. The method further comprises identifying multiple chimeric peptides, each having the chosen actual crossover point and at least one partial sequence of the reference sequence, terminating at the crossover point. The computer-implemented method for determining the fitness of two or more potential crossover points comprises identifying a first potential crossover point in a reference peptide sequence; generating a first chimeric sequence having the potential crossover point with and comprising one partial sequence from the reference peptide sequence and another partial sequence from a different sequence; applying the first chimeric sequence to a contact map for the reference peptide sequence; calculating a value of a fitness **parameter** from residue-residue interactions in the first chimeric sequence selected using the contact map; repeating the above steps for one or more additional chimeric sequences; calculating an overall fitness value from the value of the fitness **parameter** for each chimeric sequence; identifying a second potential crossover point in the reference peptide sequence; and performing the above steps for the second potential crossover point. The method comprises repeating the above steps for multiple additional potential crossover points. The method, further comprises, from the multiple potential crossover points, selecting one or more crossover points for use in one or more peptides to be produced, based on the overall fitness values for the multiple potential fitness values. Selecting crossover points between two or more biomolecules comprises: (a) providing a reference sequence of a reference biomolecule; generating a contact map for the reference sequence; (b) providing a first sequence of a first biomolecule and a second sequence biomolecule, between which one or more crossover points are determined; (c) aligning the first and second sequences with the reference sequence; and (d) replacing a subsequence from the first sequence with a subsequence from the second sequence to produce a chimeric biomolecule sequence, where the subsequences terminate at a selected crossover point; comparing the chimeric biomolecule sequence with the contact map to select two or more elements in the chimeric biomolecule sequence that correspond to proximal elements in the contact map of the reference biomolecule; and scoring the selected elements, where the score provides a measure of the likelihood of the chimeric biomolecule sequence having a **property** similar or identical to the reference biomolecule. The biomolecules comprise polypeptides or proteins and the elements comprise amino acid residues. The biomolecules comprise nucleic acids and the elements comprise nucleotides. The reference sequence is the first sequence. Generating the contact map comprises determining one or more spacings of elements in the biomolecule and identifying two or more proximal elements within a critical distance of one another. The critical distance ranges from about 2-6.5 Angstroms. The critical distance is less than about 4.5 Angstroms. Providing the first sequence and the second sequence comprises providing amino acid sequences or nucleic acid sequences for two proteins having an amino acid sequence identity of about 60% or less as determined using a BLASTP algorithm and default **parameters**. The **property** similar or identical to the reference biomolecule comprises an enzyme activity or a protein stability. The scoring comprises calculating the contact energy of the two or more selected elements in the chimeric biomolecule sequence. The contact energy is calculated using a Miyazawa-Jernigan energy matrix. The scoring comprises presenting the score in a triangular contour plot. The further comprises synthesizing one or more chimeric biomolecules. Synthesizing the one or more chimeric

biomolecules comprises providing one or more recombinant constructs. It comprises performing one or more recombination processes upon two or more parental sequences, thus generating one or more recombinant constructs encoding the chimeric biomolecule. The method further comprises assaying the one or more chimeric biomolecules. Preferred **Product**: The computer program **product** further comprises code for calculating an individual value of the fitness **parameter** for a chimera sequence having the potential crossover point trader consideration. The code for calculating the individual value of the fitness **parameter** comprises: (a) code for aligning the chimera sequence to the reference peptide sequence; (b) code for identifying contacting residues of the chimera from a contact map; and (c) code for summing residue-residue potentials for contacting residues of the chimera. The contact map is a three-dimensional arrangement of residues in the reference peptide. The **product** comprises code for choosing multiple crossover points for multiple chimeric peptides comprising partial sequences of the reference peptide sequence. It further comprises code for identifying a library of peptides comprising the multiple chimeric peptides. It comprises code for identifying multiple chimeric peptides, each having the chosen actual crossover point and at least one partial sequence of the reference sequence, terminating at the crossover point. Preferred Medium: The computer-readable medium comprises the computer code for at least one additional crossover site. The inputting comprises providing the amino acid sequence of a known biomolecule or providing the nucleic acid sequence encoding the known biomolecule. The inputting comprises querying a nucleic acid or biomolecule database. The generating a contact map comprises determining amino acid spacing from a crystallographic model or an NMR model of the reference biomolecule and identifying residues within a critical distance of each other. The generating a contact map comprises determining amino acid spacing from an protein-folding analysis of the reference biomolecule and identifying residues within a critical distance of each other. The critical distance varies with the nature of the amino acid-amino acid interaction. The critical distance is less than about 4.5 Angstroms. The aligning a first and a second amino acid sequence comprises querying a nucleic acid or protein database. The scoring comprises calculating the contact energy of a pair of amino acids in a chimeric amino acid sequence where that pair of residues corresponds to residues that are in contact in the contact map. The scoring comprises summing the contact energy for all of the residues in the chimeric amino acid sequence that correspond to residues that are in contact in the contact map. Scoring comprises calculating a contact energy of interacting residues using a Miyazawa energy matrix, presenting the score to a user in a graphical user interface, and presenting the score in a triangle plot.

USE - The method is useful for **optimizing** directed points for directed mutation. The computer program **product** is useful for determining the fitness of multiple potential crossover points on a reference peptide sequence. The integrated system is useful for assessing crossover sites. (78 pages)

L6 ANSWER 7 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 2003-17346 BIOTECHDS
TI Whole cell engineering of new or modified phenotypes using real-time metabolic flux analysis, by modifying the genetic composition of a cell and measuring a metabolic **parameter** of the cell by monitoring the cell culture in real-time;
genetically engineering cell using real-time metabolic flux for use in tissue engineering
AU FU P; SHORT J M
PA DIVERSA CORP
PI WO 2003029425 10 Apr 2003
AI WO 2002-US31380 1 Oct 2002
PRAI US 2001-337526 9 Nov 2001; US 2001-326653 1 Oct 2001
DT Patent

LA English
OS WPI: 2003-430201 [40]
AB DERWENT ABSTRACT:
NOVELTY - Whole cell engineering of new or modified phenotypes by using real-time (RT) metabolic flux analysis, involves making a modified cell (MC) by modifying genetic composition of a cell, culturing MC to generate many MCs, measuring a metabolic **parameter** of the cell by monitoring the cell culture in RT, and analyzing data to determine if measured **parameter** differs from a comparable measurement in an unmodified cell under similar conditions.

DETAILED DESCRIPTION - Whole cell engineering (M1) of new or modified phenotypes by using RT metabolic flux analysis, comprises making a MC by modifying the genetic composition of a cell, culturing the MC to generate several MCs, measuring at least one metabolic **parameter** of the cell by monitoring the cell culture in RT, and analyzing the data to determine if the measured **parameter** differs from a comparable measurement in an unmodified cell under similar conditions, therefore identifying an engineered phenotype in the cell using RT metabolic flux analysis. INDEPENDENT CLAIMS are also included for: (1) determining (M2) the optimal culture conditions for generating a desired **product** or a desired phenotype in cultured cells, by comprising culturing cells in a controllable cell environment, measuring at least one metabolic **parameter** to obtain at least two different measurements in RT during the culturing, processing the two different measurements to determine a rate of change in the metabolic **parameter** in RT during the culturing, and using the rate of change in a known metabolic network of the cells to determine a RT metabolic flux distributions in the cells during the culturing; (2) an article (I) comprising a machine-readable medium including machine-executable instructions, the instructions being operative to cause a machine to: electronically interface with several measuring devices coupled to a controllable cell environment to, in RT, obtain electronic data indicative of several metabolic **parameters** or conditions of cell culturing, process the electronic data, in RT, to produce values for a set of selected metabolic **parameters** or conditions indicative of RT metabolic **properties** of the cultured cells in the controllable cell environment, retrieve information from at least one database comprising data on a metabolic network for the cultured cells, and use the metabolic network and values for the set of selected metabolic **parameters** or conditions to determine a RT metabolic flux distribution in the cultured cells; (3) a system, comprising a controllable cell environment for culturing cells, where the operating conditions for culturing the cells is controllable in response to a control command, a sensing subsystem coupled to the controllable cell environment to obtain, in RT during the culturing, measurements associated with culturing of the cells in the controllable cell environment, and a system controller coupled to the sensing subsystem to receive, in RT during the culturing, the measurements and operable to process the measurements to produce a RT metabolic flux distribution in the cultured cells; (4) controlling a computer to perform an on-line metabolic flux analysis for cells under culturing in RT; (5) a cell made by (M1); (6) a cultured cell system having optimal culture conditions for generating a desired **product** or a desired phenotype made by (M2); (7) identifying proteins by differential labeling of peptides, or defining the expressed proteins associated with a given cellular state; (8) quantifying changes in protein expression between at least two cellular states; (9) a chimeric labeling reagent by a first domain comprising a biotin, and a second domain comprising a reactive group capable of covalently binding to an amino acid, where the labeling reagent comprises at least one isotope; (10) comparing relative protein concentrations in a sample; and (11) a multidimensional micro liquid chromatography MS/MS (microLC-MS/MS) system (II) comprising three-dimensional (3-D) microcapillary columns for liquid chromatograph (LC) separation of peptides comprising a configuration comprising a

reverse phase (RP1) chromatograph, a strong cation exchange (SCX) chromatograph and a reverse phase (RP2) resin chromatograph.

BIOTECHNOLOGY - Preferred Method: The genetic composition of the cell is modified by addition of a nucleic acid to the cell, deletion of a sequence or modification of a sequence in the cell, or by modifying or knocking out the expression of a gene. The nucleic acid comprises a nucleic acid heterologous or homologous to the cell. The homologous nucleic acid comprises a modified homologous nucleic acid or gene. (M1) further comprises selecting a cell comprising a newly engineered phenotype and culturing the selected cell, thus generating a new cell strain comprising a newly engineered phenotype. (M1) comprises isolating a cell comprising a newly engineered phenotype, preferably stable or inducible phenotype. Modifying the genetic composition of a cell comprises insertion of a construct into the cell, where the construct comprises a nucleic acid operably linked to a constitutively active or inducible promoter. The nucleic acid added to the cell is stably inserted into the genome of the cell, propagates as an episome in the cell or encodes a polypeptide comprising a modified homologous polypeptide or heterologous polypeptide. The nucleic acid added to the cell encodes a transcript comprising a sequence that is antisense to a homologous transcript. Modifying the genetic composition of the cell comprises increasing or decreasing the expression of an mRNA transcript or polypeptide. Modifying the homologous gene comprises knocking out or increasing expression of the homologous gene. The heterologous gene comprises a sequence modified homologous gene, where the sequence modification is made by providing a template polynucleotide comprising a homologous gene of the cell, providing several oligonucleotides, where each oligonucleotide comprises a sequence homologous to the template polynucleotide, thus targeting a specific sequence of the template polynucleotide, and a sequence that is a variant of the homologous gene, and generating progeny polynucleotides comprising non-stochastic sequence variations by replicating the template polynucleotide with the oligonucleotides, thus generating polynucleotides comprising homologous gene sequence variations. Alternatively, the method comprises combining a building block polynucleotide with a template polynucleotide such that the building block polynucleotide cross-over reassembles with the template polynucleotide to generate polynucleotides comprising homologous gene sequence variations. The building block polynucleotides are designed to cross-over reassemble with the template polynucleotide at a predetermined sequence, and comprises a sequence that is a variant of the homologous gene and a sequence homologous to the template polynucleotide flanking the variant sequence. The measured metabolic **parameter** comprises: (a) rate of cell growth which is measured by a change in optical density of the culture; (b) a change in the expression of a polypeptide which is measured by one-dimensional gel electrophoresis, two-dimensional gel electrophoresis, tandem mass spectrography, radioimmunoassay (RIA), ELISA, immunoprecipitation or a Western blot; (c) a change in expression of at least one transcript measured by hybridization, quantitative amplification or Northern blot, or the expression of transcript of a newly introduced gene. The transcript expression is measured by hybridization of a sample comprising transcripts of a cell or nucleic acid representative of or complementary to transcripts of a cell by hybridization to immobilized nucleic acids on an array; (d) an increase or a decrease in a secondary metabolite, preferably glycerol, ethanol and/or methanol, or an increase or a decrease in an organic acid such as acetate, butyrate, succinate, oxaloacetate, fumarate, alpha- ketoglutarate and/or phosphate; (e) an increase or a decrease in intracellular pH or in synthesis of DNA over time, measured by intracellular application of a dye, and the change in fluorescence of the dye is measured over time; (f) an increase or a decrease in uptake of a composition, preferably a metabolite such as monosaccharide, disaccharide, polysaccharide, lipid, nucleic acid, amino acid or polypeptide, more preferably antibiotic, metal, steroid or antibody; or (g) an increase or a decrease in the secretion of a

byproduct or a secreted composition of a cell, preferably toxin, lymphokine, polysaccharide, lipid, nucleic acid, amino acid, polypeptide or an antibody. The RT monitoring simultaneously measures several metabolic **parameters** by using a cell growth monitor device such as Wedgewood Technology, Inc., cell growth monitor model 652. The RT simultaneous monitoring measures uptake of substrates, levels of intracellular organic acids and levels of intracellular amino acids, cell density, uptake of glucose, levels of acetate, butyrate, succinate, oxaloacetate, fumarate, alpha-ketoglutarate, phosphate and/or levels of intracellular natural amino acids. (M1) further comprises use of a computer-implemented program to RT monitor the change in measured metabolic **parameters** over time. The computer-implemented method comprises metabolic network equations, pathway analysis and the program comprises a preprocessing unit to filter out the errors for the measurement before the metabolic flux analysis. (M2) further comprises adjusting an operating **parameter** of the controllable cell environment based on the determined RT metabolic flux distribution to change a culturing condition to modify the metabolic flux distribution during the culturing, thus **optimizing** culture conditions for generating a desired **product** or a desired phenotype. Preferred Article: The data on the metabolic network for the cultured cells comprises a stoichiometry matrix for the cultured cells. The matrix comprise representation of a metabolic network of the cultured cells and defines the presence or absence of metabolic pathway associations. The metabolic network for the selected cell comprises a set of stoichiometric equations for metabolites in the selected cell. The instructions are further operative to cause the machine to present the RT metabolic flux distribution in the selected cell in a display device coupled to the machine.

USE - (M1) is useful for whole cell engineering of new or modified phenotypes by using RT metabolic flux analysis. The cell is a bacterial, fungal, yeast, plant, insect cell, mammalian, preferably human cell. The newly engineered phenotype is an increased or decreased expression or amount of a polypeptide, mRNA transcript, expression of a gene, resistance or sensitivity to a toxin, use or production of a metabolite, uptake of a compound by the cell, rate of metabolism or growth rate. (I) is useful for determining a RT metabolic flux distribution in the cultured cells. (II) is useful for separating peptides (claimed).

EXAMPLE - Metabolic flux analysis of a culture of the yeast Saccharomyces cerevisiae was determined. The yeast S. cerevisiae strain ATCC S288C was used in the experiment. SD medium was made with 0.16% yeast nitrogen base (YNB) without amino acids and hexose (BIO101), 0.5% ammonium sulfate, supplemented with 2% glucose. Cultures were all grown at 30 degreesC. A 15 ml sterile test tube containing 5 ml of SD media was inoculated with a colony from a streaked YPD plate. The yeast culture was grown overnight in a shaking incubator. The primary seed was transferred to a 1 l Erlenmeyer shake flask containing 250 ml of pre-warmed SD medium. The culture was grown approximately 12 hours in the same shaking incubator before being used as the secondary seed. The secondary seed was used to inoculate a 5 l bioreactor. BIOFLO 3000 (RTM) had its own controllers for temperature, pH and dissolved oxygen (DO). The S. cerevisiae cultivation process was monitored and controlled automatically using a PENTIUM II (RTM) equipped with a computer interface board. The data acquisition and process control program was written in LabVIEW6.0. The analog outputs of the analyzers were connected to the data acquisition board AT-MIO-16E-10. The temperature was controlled using a circulating water bath with a temperature control module. During the cultivation period, samples were taken periodically for off-line analysis. Aliquots at 2 ml volumes were withdrawn rapidly from the fermentor, minimizing perturbations to their environment. The samples were then used to determine cell, glucose, ethanol, acetate and organic acid concentrations. Cellular growth was monitored by measuring the optical density (OD) at 600 nm and 660 nm with DU 7400 Spectrophotometer. Concentrations of glucose and ethanol were determined using YSI2700

SELECT BIOCHEMISTRY (RTM) analyzer. The concentrations of other metabolites in the culture media were determined by HPLC. An aminex HPX-87H (RTM) ion exchange carbohydrate-organic acid column at 65degreesC was used with degassed 5 mM sulfuric acid as the mobile phase and UV detection. The metabolic flux analysis results for this S. cerevisiae system was summarized as 2.489 acetyl coenzyme A (ACCOA) + 11.418 NADPH + 1.572 NAD = BIOMAS + 1.572 NADH + 1.271 + CO2 + 11.418 NADP. (174 pages)

L6 ANSWER 8 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 2003-13324 BIOTECHDS
TI Parallel culture of microorganisms, useful e.g. for **optimizing** culture media, comprises preparing many microcultures and addition of nutrients, effectors and metabolites;
fungus culture fermentation useful for biotechnology and microbiology
AU HILLIGER M; MUELLER P; ROTH M; GROTH I; KUMMER C; MARTIN K; SCHROECKH V; METZE J; KOEHLER M; HENKEL T; GASTROCK G; LEMKE K
PA KNOELL-INST NATURSTOFF-FORSCH EV HANS; INST BIOPROZESS and ANALYSENMESSTECHNIK EV; INST PHYSIKALISCHE HOCHTECHNOLOGIE EV; HILLIGER M; HILLIGER S
PI WO 2003025113 27 Mar 2003
AI WO 2002-DE3451 13 Sep 2002
PRAI DE 2001-1045568 14 Sep 2001; DE 2001-1045568 14 Sep 2001
DT Patent
LA German
OS WPI: 2003-313347 [30]
AB DERWENT ABSTRACT:
NOVELTY - Parallel culture (M) of microorganisms (A) comprising adding nutrients, effectors and/or microbial metabolites to a suspension or culture, free of coarse solids, of a homogeneous or heterogeneous microbial population, is new.

DETAILED DESCRIPTION - M comprises adding nutrients, effectors and/or microbial metabolites to a suspension or culture, free of coarse solids, of a homogeneous or heterogeneous microbial population. A volume (v) of the suspension, containing N microorganisms, is divided into n1 (= N to 100, preferably N-10N) portions and each portion (optionally with addition of nutrients, effectors and/or microbial metabolites), used to inoculate n2 separate microcultures, in microareas or microcavities, where n2 is at least equal to n1. The microcultures are incubated, optionally with addition of nutrients, effectors and/or microbial metabolites, and the physiological **parameters** and growth of the individual microcultures are determined.

USE - M is useful in biotechnology; genetics; (medical) microbiology; pharmaceuticals and food/environmental microbiology, e.g. for: (i) **optimization** of culture media; (ii) screening to identify new natural **products** and specific metabolic **properties**; (iii) quantitative and qualitative determination of the effects of nutrients, effectors and/or microbial metabolites; and (iv) selection of clones with specific **properties**, from a large population, e.g. one produced by mutagenesis or gene transfer.

ADVANTAGE - M allows isolation of individual cells with selected **properties**, also their characterization and subsequent culture in pure form, and determination of the effects of variables on fully comparable cultures. The number of individual cultures that can be examined is much greater than in known methods.

EXAMPLE - Saccharomyces cerevisiae was cultured in an essentially standard medium, then plated at 25 cell/cm2 on to agar, cultured, then silicone punches used to transfer cells from the agar to nanotiter plates cavities filled with nutrient medium. The plates were incubated at 30degreesC, with turbidimetric monitoring of cell growth. Clones were isolated from selected cultures, using a sterile needle to rupture the membrane at the base of the plate. (17 pages)

L6 ANSWER 9 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 2003-14439 BIOTECHDS

TI Producing proteins with a predetermined **property** comprises introducing nucleic acids encoding a modified target protein into host cells and expressing and screening for proteins with different activity than the target protein;
vector-mediated gene transfer and expression in cell array for recombinant protein production, virus titer and high throughput screening

AU VEGA M; DRITTANTI L; FLAUX M
PA NAUTILUS BIOTECH

PI WO 2003023032 20 Mar 2003
AI WO 2002-IB3921 16 Aug 2002
PRAI US 2001-22249 17 Dec 2001; US 2001-315382 27 Aug 2001
DT Patent
LA English
OS WPI: 2003-354538 [33]

AB DERWENT ABSTRACT:
NOVELTY - Producing (M1) a peptide, polypeptide, or protein having a predetermined **property**, comprises producing a population of sets of nucleic acid molecules that encode modified forms of a target protein; introducing each set of nucleic acids into host cells and expressing the encoded protein, where the host cells are present in an addressable array; and individually screening the sets of encoded proteins.

DETAILED DESCRIPTION - Producing (M1) a peptide, polypeptide, or protein having a predetermined **property**, comprises producing a population of sets of nucleic acid molecules that encode modified forms of a target protein; introducing each set of nucleic acids into host cells and expressing the encoded protein, where the host cells are present in an addressable array; and individually screening the sets of encoded proteins to identify one or more proteins that have activity that differs from the target protein, where each such protein is designated a hit.

BIOTECHNOLOGY - Preferred Method: In (M1), each set of nucleic acid molecules is individually designed and synthesized, and is deposited at a locus in an addressable array. Each polynucleotide in a set encodes a protein that differs by at least one amino acid from the target protein. The array comprises a solid support with loci or wells for containing or retaining cells, and each locus or well contains one set of cells. The nucleic acid molecules comprise viral vectors, and the cells are eukaryotic cells that are transduced with the vectors. The nucleic acids comprise plasmids and the cells are bacterial cells. The method further comprises modifying the nucleic acid molecules that encode the hits, to produce a set of nucleic acid molecules that encode modified hits; introducing each set of nucleic acids that encode the modified hits into cells; and individually screening the sets of cells that contain the nucleic acid molecules to identify one or more cells that encode(s) a protein that has activity that differs from the target protein and has **properties** that differ from the original hits, where each such protein is designated a lead. The nucleic acid molecules that encode modified forms of a target protein or that encode the hits, are produced by nucleic acid shuffling, recombination, site-directed or random mutagenesis, or de novo synthesis. These nucleic acids are also produced by systematically changing each codon in the target protein to a pre-selected codon, or by systematically replacing each codon that is a hit, with a codon encoding the remaining amino acids, to produce nucleic acid molecules each differing by at least one codon and encoding modified hits to identify leads. The codon is selected from a codon encoding Ala (A), Ser (S), Pro (P), Gly (G), Arg (R), Asn (N), Asp (D), Cys (C), Gln (Q), Glu (E), His (H), Ile (I), Leu (L), Lys (K), Met (M), Phe (F), Thr (T), Trp (W), Tyr (Y) or Val (V). In addition, the method comprises the steps of recombining the nucleic acid molecules encoding the leads, introducing those nucleic acid molecules into cells, and screening the cells to identify nucleic acid molecules that encode **optimized** leads. The recombining is two, three or more up to all of the nucleic

acids encoding the leads. The recombining step is effected by nucleic acid shuffling, recombination, site-directed or random mutagenesis or de novo synthesis. The modifications are effected in a selected domain of the target protein or along the full length of the target protein. The change in activity is at least about 10, 20, 30, 40, 50, 75, 100, 200, 500 or 1000%. In the step of introducing each set of nucleic acids into host cells, the titer of the viral vectors in each set of cells is assessed. Titering is effected by real time virus titering, which comprises: (a) incubating the nucleic acid molecules or a vector (biological agent) comprising the nucleic acid molecules at an initial concentration (C), which is the unknown titer, with the host cells at a constant known concentration (D); (b) measuring at successive times, an output signal, i; (c) determining the time (tbeta), where: tbeta corresponds to i = beta; betamin is less than beta is less than betamax; betamin and betamax correspond to values of i at the inflection point of the curve i = f (t), for the minimal and maximal values, respectively, of the concentrations of a reference biological agent for which the curve tbeta = f (c) is predetermined; and (d) determining the initial concentration (C). Titering is also effected by Tagged Replication and Expression Enhancement, which comprises incubating with host cells a reporter virus vector with a titering virus of unknown titer, where the titering virus increases or decreases the output signal from the reporter virus; measuring the output signal of the reporter virus and determining the titer of the reporter virus; and determining the titer of the interfering virus by comparing the titer of the reporter virus in the presence and absence of the interfering virus. In the step of individually screening the sets of cells, the titer of the viral vectors in each set of cells is determined. The target protein is a protein involved in viral replication. The performance of the screened proteins is evaluated by Hill analysis or by fitting the output signal to a curve representative of the interaction of the target protein and a test compound. The Hill analysis comprises: (a) preparing a sample of each nucleic acid molecule or a plasmid or vector that comprises each nucleic acid molecule (biological agent), where each sample is obtained by a serial dilution of the molecules or vector or plasmid at a concentration (R1); (b) incubating each sample of the dilution obtained in (a) with the host cells (target cells) at a constant concentration (R2); (c) determining a (P) **product** from the reaction R1 + R2, at a (t) moment, in each the sample; and (d) preparing a theoretical curve (H) from the experimental points R1 and P, for each biological agent by iterative approximation of **parameters** of the reaction R1 + R2 right arrow P, at the t moment, in accordance with the equation: P = Pmax (piR1)r / (K + (piR1)r) r = 1, ..., n (2) where: R1 represents the biological agent concentration in a sample from the scale; R2 is concentration of target cells (in vitro or in vivo); P (output) represents the **product** from the reaction R1 + R2 at a t moment; Pmax represents the reaction maximal capacity; K represents, at a constant R2 concentration, the biological system for responding to the biological agent (resistance constant R2); r represents a dependent coefficient of R1 and corresponds to the Hill coefficient; and pi represents the intrinsic power of the R1 biological agent to induce a response in the biological system (P production at the t moment); and (e) sorting the K and pi values obtained in (d) for each protein encoded by the nucleic acid molecules or plasmids or vectors and the cells, and then ranking according to the their values. The process of producing a peptide, polypeptide, or protein having a predetermined **property** , is automated and is computer-controlled. Alternatively, the non-random method for generating proteins with a desired **property**, comprises identifying a target protein; preparing sets of variant nucleic acid molecules that each encode a protein that differs by one amino acid from the target protein; screening and selecting the sets of variant nucleic molecules to identify those that encode proteins that have activity that differs by a predetermined amount from the activity of the target protein, thus, identifying proteins that are hits; identifying the

residues in the hit proteins encoded by the variant nucleic acid molecules that differ from the target proteins; preparing further sets of variant nucleic acid molecules in which each codon in the nucleic acid molecule encoding each of the identified residues in each of the hits is replaced with codons encoding each of the remaining 18 amino acids to produce the further sets of variant nucleic acids, where each set differs from other set by one codon; and screening the further sets of nucleic acid molecules to identify those that encode proteins that have activity that differs from the activity of the hits, thus, identifying nucleic acid molecules that encode leads. The replaced amino acid positions comprise a functional domain of the protein. The positions in the protein in which amino acids are replaced comprise at least about 50, 90 or 95% or all of the amino acids in the protein. Each set of nucleic acid molecules is generated, processed and screened separately or in parallel. The method for producing a protein having modified **properties**, may also comprise preparing a population of nucleic acid molecules that encode rationally modified proteins, inserting the population into expression vectors, introducing each vector into host cells and expressing the modified proteins, screening each modified protein, and selecting one or more that has (have) a modified **property**.

USE - The method is useful in performing high throughput directed evolution of peptides and proteins, particularly those that act in complex biological settings, by rational mutagenesis. The method is also used for generating protein variants and for titering viruses. (71 pages)

L6 ANSWER 10 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 2003-11295 BIOTECHDS
TI Generating computationally prescreened secondary protein libraries, by receiving scaffold protein with residue positions, and analyzing the interaction of rotamers/amino acids with protein to generate protein sequences;
bioinformatic hardware and bioinformatic software for protein library screening and prescreening
AU BENTZIEN J; DAHIYAT B I; DESJARLAIS J; HAYES R J; VIELMETTER J
PA XENCOR
PI WO 2003014325 20 Feb 2003
AI WO 2002-US25588 12 Aug 2002
PRAI US 2002-352103 25 Jan 2002; US 2001-311545 10 Aug 2001
DT Patent
LA English
OS WPI: 2003-256574 [25]
AB DERWENT ABSTRACT:

NOVELTY - A method (M1) executed by a computer under the control of a program, involves receiving a scaffold protein structure with residue positions (RPs), selecting a collection of variable RPs, establishing a group of potential rotamers or amino acids (aa), and analyzing the interaction of each of the rotamers or (aa)s, with the remainder of the protein to generate a set of **optimized** protein sequences.

DETAILED DESCRIPTION - The method executed by a computer under the control of a program, the computer including a memory for storing the program, involves: (a) receiving a scaffold protein structure with residue positions; (b) selecting a collection of variable residue positions from the residue positions; (c) establishing a group of potential rotamers or (aa)s, for each of the variable residue positions, and where a first group for a first variable residue position has a first set of at least two different (aa) side chains, or rotamers from two different (aa) side chains, and where a second group for a second variable residue position has a second set of at least two different (aa) side chains, or rotamers from two different (aa) side chains; and (d) analyzing the interaction of each of the rotamers in each group or (aa)s, with all or part of the remainder of the protein to generate a set of **optimized** protein sequences. Alternatively, the method involves providing a sequence alignment of several related proteins, generating frequencies of occurrence for individual (aa)s in at a least several of

positions with the alignments, or proteins, creating a pseudo-energy scoring function using the frequencies, and using the pseudo-energy scoring function and at least one additional scoring function, to generate a set of **optimized** protein sequences. INDEPENDENT CLAIMS are also included for the following: (1) generating (M2) a secondary library of scaffold protein variants, by providing a primary library comprising a filtered set of scaffold protein primary variant sequences, generating a list of primary variant positions in the primary library or a probability distribution of (aa) residues in several of variant positions, combining several of the primary variant positions or (aa) residues to generate a secondary library of secondary sequences; or providing a first library rank-ordered list of scaffold protein primary variants, generating (aa) residues, and synthesizing scaffold protein secondary variants; (2) a composition comprising several secondary variant proteins comprising a subset of the secondary library; (3) a composition comprising several nucleic acids encoding the above secondary variant proteins; (4) identifying (M3) proteins that have a similar conformation to a target protein, by receiving at least one scaffold protein structure with variable residue positions of a target protein, computationally generating a set of primary variant (aa) sequences that adopt a conformation similar to the conformation of the target protein, and identifying at least one protein sequence that is similar to at least one member of the set of primary variants, but is dissimilar to the target protein (aa) sequence; and (5) generating (M4) variant protein sequence libraries, by providing populations of at least two double stranded donor fragments corresponding to a nucleic acid template, adding polymerase primers capable of hybridizing to end regions of each of the population of donor fragments, generating a population of hybrid double stranded molecules where one strand comprises a 5'-purification tag and the other strand comprises a 5'-phosphorylated overhang, enriching for variant strands by removing strands comprising a 5'-biotin moiety, annealing the variant strands to form at least two double stranded ligation substrates, and ligating the ligation substrates to form a double stranded ligation **product** where the ligation **product** encodes a variant protein.

BIOTECHNOLOGY - Preferred Method: The first and second sets of rotamers are same or different. After the generation of the **optimized** protein sequences, a library of the **optimized** protein sequences is generated. The method further involves physically generating at least one member of the set of **optimized** protein sequences and experimentally testing the sequences for a desired function. The frequencies are weighted using a diversity, sequence homology, structural homology weighting function, weighting function based on physical **properties**, or functional-based weighting function. The homology is high, the weighting is high, or low. The multiply sequence alignment comprises proteins with related three-dimensional structures. The pseudo-energy is based on logarithms of the frequencies, and the pseudo-energy scoring function is based on log-odds ratios. The computational method used to generate the primary library is Protein Design Automation (PDA) technology. Analyzing interactions of the rotamers with each other and with the protein backbone to generate **optimized** protein sequences involves the use of number of scoring functions to calculate energies of the rotamer interactions, with the backbone and with other rotamers. The (aa)s with a frequency of occurrence of at least 1%, 5%, 10%, or 20% are selected. Analyzing further involves at least two scoring functions. The scoring function is selected from van der Waals potential scoring function, a hydrogen bond potential scoring function, an atomic solvation scoring function, an electrostatic scoring function, a secondary structure propensity scoring function and a pseudo-energy scoring function. The method also provides an (aa) substitution matrix selected from PAM, BLOSUM, and DAYHOFF. The results of PDA technology calculations is performed on decoy structures which is used to obtain optimal sets of scoring function weights. A set of **parameters** or weights are

used in conjunction with a protein design algorithm to design one or more protein sequences and structures. These generated structures are then treated as decoy structures. The calculated energies of each decoy and comparison of each of its energy components to the reference, is used to repeatedly **optimize** the **parameters**. The weights are **optimized** on a set of the scaffold proteins. The extent of increase or decrease of the weight is based on the total Boltzmann probabilities of the reference and decoy states. The extent of increase or decrease of the weight is based on the difference between individual scores of the decoy and reference states. The method further involves replacing at least a single (aa) in the scaffold protein to create a variable sequence and analyzing the variable sequence using the scoring functions. Further comprising replacing a subset of (aa)s, the subset selected from core, boundary, and surface (aa)s. Further comprising protein design automation or sequence prediction algorithm. In alternative methods, the rotamer internal energies are included, the energies or scores that are a function of the conformation and/or identity of 3 or more (aa)s are included. The energy increase is applied to several of (aa) positions. The energy increase includes the energy increase of a set of rotamers for at least one (aa) positions, or several of (aa) positions. The protein design cycle comprises applying protein design automation technology, sequence prediction algorithm, or a force field calculation. The method is also carried out using clustering algorithm and taboo search. The clustering algorithm comprises a single-linkage, complete linkage, or an average linkage clustering algorithm. The subsets are clustered according to sequence similarity, or energetic similarity. The DNA shuffling is applied with the subsets to generate a library of **optimized** protein sequences. The subsets are used to generate secondary libraries comprising related sequences. A single linkage clustering algorithm is used to form subsets directly from the **optimized** protein sequences. The measure of similarity between two sequences are based on standard sequence similarity comparison, where the similarity scores include BLOSUM similarity score. M2 further involves synthesizing several of the secondary sequences. The synthesizing is done by multiple PCR with pooled oligonucleotides. The pooled oligonucleotides are added in equimolar amounts, or in amounts that correspond to the frequency of the mutation. M3 further involves confirming that the protein will adopt the conformation of the target protein. An (aa) sequence with less than 30%, or less than 20% sequence identity is dissimilar. A similar conformation is a protein comprising a position for a given fold. The computationally generating is applying a protein design algorithm, or automation. The computationally generating step comprises a taboo search. The computationally generating step involves applying a sequence prediction algorithm, and are used to create a Position Specific Scoring Matrix. The computationally generating includes the use of at least two scoring functions. The method for identifying the protein comprises searching public databases, or using a dynamic programming algorithm. The confirming is selected from x-ray crystallography, NMR spectroscopy, and their combinations. In M4, one of the polymerase primers and the nucleic acid template generates a variant nucleic acid strand. The first three steps are repeated to generate a variant protein.

USE - M1 is useful for generating an **optimized** protein sequences using a computer under the control of a program (claimed), and also in protein fold identification. M1 is also useful for computational screening of random peptide libraries for the purpose of target identification, and for compounds that are capable of inducing specific alterations in cellular physiology or phenotype leading to the discovery of proteins that function in a variety of biochemical and signal transduction pathways. M1 is further useful for generating secondary libraries of scaffold protein variants.

ADVANTAGE - M1 is efficient, and rapid.

EXAMPLE - Computational prescreening on beta-lactamase TEM-1. Brookhaven Protein Data Bank entry 1BTL was used as the starting

structure. All water molecules and the sulfate group were removed and explicit hydrogen were generated on the structure. The structure was then minimized for 50 steps. This minimized structure served as the template for all the protein design calculations. Computational screening of sequences was performed using Protein Design Automation (PDA) technology. A 4Angstrom sphere was drawn around the heavy side chain atoms within this distance cutoff were selected. This yielded the following 7 positions such as F72, Y105, N132, N136, L169, N170, and K234. Two of these residues, N132 and K234, are highly conserved across several different beta-lactamases and were therefore not included in the design, leaving five variable residue positions (F72, Y105, N136, L169, N170). These designed positions were allowed to change their identity to any of the 20 naturally occurring (aa)s except proline, cysteine, and glycine (a total of 17 (aa)s). Proline was usually not allowed since it was difficult to define appropriate rotamers for proline, cysteine was excluded to prevent formation of disulfide bonds, and glycine was excluded because of conformational flexibility. The Dead End Elimination (DEE) **optimization** method was used to find the lowest energy, ground state sequence. DEE cutoffs of 50 and 100 kcal/mol were used for singles and doubles energy calculations, respectively. Starting from the DEE ground state sequence, a Monte Carlo (MC) calculation was performed that generated a list of the 1000 lowest energy sequences. The MC **parameters** were 100 annealing cycles with 1000000 steps per cycle. The non-productive cycle limit was set to 50. The following probability distribution was then calculated from the top 1000 sequences in the MC list. It showed the number of occurrences of each of the (aa)s selected for each position. This probability distribution was then transformed into a rounded probability distribution. The computational pre-screening resulted in an enormous reduction in the size of the problem. Originally, 17 different (aa)s were allowed at each of the 5 designed positions. This was pared down to just 210 possible sequences a reduction of nearly four orders of magnitude. (158 pages)

L6 ANSWER 11 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 2003-05761 BIOTECHDS
TI **Optimization** of isonovalal production from alpha-pinene oxide using permeabilized cells of Pseudomonas rhodesiae CIP 107491; bacterium fermentation having alpha-pinene-oxide-decyclase activity
AU FONTANILLE P; LARROCHE C
CS Univ Clermont Ferrand
LO Larroche C, Univ Clermont Ferrand, CUST, Lab Genie Chim and Biochim, BP 206, F-63174 Aubiere, France
SO APPLIED MICROBIOLOGY AND BIOTECHNOLOGY; (2003) 60, 5, 534-540 ISSN: 0175-7598
DT Journal
LA English
AB AUTHOR ABSTRACT - **Optimization** studies on the synthesis of isonovalal from alpha-pinene oxideby Pseudomonas rhodesiae CIP 107491 operated in a biphasic medium are presented. Three key **parameters** are identified. The first is the need for a permeabilization of cells by freezing them and then treating the thawed material with an organic solvent such as chloroform, toluene or diethyl ether. This operation allows both enzyme release into the aqueous phase outside thecells and an improvement in the transport **properties** of both substrate andproduct across the cell membrane, strongly increasing reaction rates. The second is that the enzyme a-pinene oxide lyase, which exhibits an irreversible inactivation by isonovalal (or a by-**product**), presents a constant turn-over, i.e., the total **product** synthesis is proportional to the biomass loading and is close to 108 mmol (16.4 g) isonovalal l(-1) g(-1) biomass. The third phenomenon is that the biphasic system used is not phase-transfer-limited, a feature attributed to the spontaneous formation of an oil-in-water emulsion. It is thus possible to carry out a very efficient process, allowingthe recovery of 2.63 mol isonovalal l(-1) (400 g l(-1)) from 25 g biomassl(-1) in 2.5 h,

corresponding to an average reaction rate as high as 0.70 mmol min(-1) g(-1) cells (160 g l(-1) h(-1)). (7 pages)

L6 ANSWER 12 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 2003-00463 BIOTECHDS
TI Sulfated polysaccharides from red microalgae have antiinflammatory **properties** in vitro and in vivo;
for use as an antiinflammatory and in the cosmetic industry
AU MATSUI MS; MUIZZUDDIN N; ARAD S; MARENUS K
CS Estee Lauder Co; Ben Gurion Univ Negev
LO Matsui MS, Estee Lauder Co, Biol Res Div, 125 Pinelawn Rd, Melville, NY 11747 USA
SO APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY; (2003) 104, 1, 13-22 ISSN: 0273-2289
DT Journal
LA English
AB AUTHOR ABSTRACT - The primary goal of the present research was to determine whether sulfatedpolysaccharides derived from red microalgae possess antiinflammatory **properties** when directed against specific **parameters** of human skin inflammation.These unique biopolymers were studied in both in vitro and in vivo models of skin inflammation. Human subjects were recruited to participate in a study in which the polysaccharide material was applied topically and shown to inhibit cutaneous erythema induced by a known irritant. Leukocyte migrationfrom capillary blood into sites of inflammation is an essential component of the inflammatory process and occurs in a series of steps, two of which are adhesion and chemotaxis. In vitro, the polysaccharide material primarilyinhibited the migration of polymorphonuclear leukocytes (PMNs) toward a standard chemoattractant molecule and also partially blocked adhesion of PMNsto endothelial cells. The data obtained strongly suggest that sulfated polysaccharides derived from red microalgae have significant beneficial potential for use in topical **products**.
In addition, the data suggested that the antiinflammatory mechanism for the polysaccharide was, at least in part, dueto inhibition of circulating immune cell recruitment toward inflammatory stimuli. (10 pages)

L6 ANSWER 13 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 2003-12390 BIOTECHDS
TI Determining optimal hybridization **parameters** for nucleic acid, useful for detecting immunoregulatory genes, comprises depositing control and test RNA on same membrane;
hybridization **parameter** determination involving macrophage-like cell, fibroblast or other immune-competent cell RNA analysis
AU HAUSMANN E H S
PA IBFB GMBH PRIVATES INST BIOMEDIZINISCHE
PI DE 10018036 28 Feb 2002
AI DE 2000-1018036 11 Feb 2000
PRAI DE 2000-1018036 11 Feb 2000; DE 2000-1018036 11 Feb 2000
DT Patent
LA German
OS WPI: 2003-269634 [27]
AB DERWENT ABSTRACT:
NOVELTY - Determining nucleic acid hybridization **parameters** using RNA (I) of activated and non-activated macrophage-like cells, fibroblasts or other immune-competent cells, immobilized on a membrane, is new.
DETAILED DESCRIPTION - Determining nucleic acid hybridization **parameters** using RNA (I) of activated and non-activated macrophage-like cells, fibroblasts or other immune-competent cells, immobilized on a membrane. The new feature is that total RNA (Ia) from an activated macrophage-like cell line, with minimal or compressed gene expression kinetics, and purified mRNA and purified rRNA are fixed to a single membrane, e.g. of nylon, and the **parameters** determined

specifically with labeled RNA or DNA probes. INDEPENDENT CLAIMS are also included for the following: (1) Test membrane for induced gene control Northern blotting comprises a nylon or nitrocellulose membrane on which have been deposited, sequentially, negative and positive controls of a compressed total RNA-kinetic activated cell or tissue, and purified rRNA and purified polyA RNA; and (2) System for induced gene control Northern blotting that includes the membrane of (1).

BIOTECHNOLOGY - Preferred Process: This detects new regulatory genes, without false interpretation caused by cross-hybridization to rRNA; splice **products**; immunoregulatory genes (expressed by macrophages, tissue or organs during immune or anti-tumor responses, or wound healing); gene regulation and cross-hybridization; and the proper orientation of subcloned DNA fragments (for expression of antisense sequences). Preferred Materials: The control RNA is isolated from permanent macrophage/monocyte cell lines, activated by endo- or exo-toxins or bacterial superantigens, so that immune-stimulated genes which depend on lipopolysaccharide (LPS) stimulation (particularly the LPS from Escherichia coli serotype OIII:B4) are transcribed. Especially genes induced by interleukins, cytokines, chemokines, growth factors, stress and heat-shock are transcribed. Subcloned gene fragments are preselected/screened for cross-hybridization to determine the most appropriate probes. Suitable sources for total RNA are the murine J774A and human THP-1 cell lines. Preferred Blot: The system of (2) contains: (i) the membrane of (1); (ii) either RNA of LPS-activated macrophages or macrophage-like cell lines as positive control, or RNA from selected cell lines that have been stimulated with toxins, superantigens etc.; and (iii) total RNA, mRNA and rRNA. The RNAs serving as positive and negative controls may be used as pellets, rather than fixed to the membrane.

USE - The method is used to determine optimal hybridization conditions for detection of immunoregulatory genes. The membrane and system are useful for induced gene control Northern blotting (claimed).

ADVANTAGE - The method allows simple determination of hybridization **properties** of RNA. The RNA is not degraded; the method can be used where gene expression is low or very little starting material is available; gene expression can be induced; probes can be preselected for specificity; and the need to screen fetal calf serum and culture media for endotoxins is avoided. All controls and test materials are present on the same membrane.

EXAMPLE - To determine hybridization and washing conditions for detection of JE/MCP-1 (monocyte chemoattractant protein-1) and interpretation of the bands, total RNA from the murine cell line J774.4 was hybridized to a specific probe at suboptimal temperature and washed. Because of cross-hybridization with rRNA, the stringency was increased gradually until the optimum was found. Optimal conditions were hybridization at 62degreesC and last wash, 5 minutes at 82degreesC. (9 pages)

L6 ANSWER 14 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 2002-15627 BIOTECHDS
TI Factors affecting the yield and **properties** of bacterial cellulose;
cellulose production, characterization and **optimization** using a horizontal fermentor
AU KRYSTYNOWICZ A; CZAJA W; WIKTOROWSKA-JEZIERSKA A; GONCALVES-MISKIEWICZ M; TURKIEWICZ M; BIELECKI S
CS Tech Univ Lodz
LO Bielecki S, Tech Univ Lodz, Inst Tech Biochem, Stefanowskiego 4-10, PL-90924 Lodz, Poland
SO JOURNAL OF INDUSTRIAL MICROBIOLOGY and BIOTECHNOLOGY; (2002) 29, 4, 189-195 ISSN: 1367-5435
DT Journal
LA English
AB AUTHOR ABSTRACT - Acetobacter xylinum E-25 has been applied in our studies in order to find optimal culture conditions for effective

bacterial cellulose (BC) production. The strain displays significantly higher stability in BC production understationary culture conditions. In contrast, intensive agitation and aeration appear to drastically reduce cellulose synthesis since such conditions induced formation of spontaneous cellulose nonproducing mutants (Cel-), which dominated in the culture. Mutation frequency strictly depends on the medium composition in agitated cultures. Enrichment of the standard SH and Yamanaka media with 1% ethanol significantly enhanced BC production in stationary cultures. Horizontal fermentors equipped with rotating discs or rollerswere successfully applied in order to improve culture conditions. Relatively slow rotation velocity (4 rpm) and large surface area enabling effectivecell attachment are optimal **parameters** for cellulose production. Physical **properties** of BC samples synthesized either in stationary cultures or in a horizontal fermentor revealed that cellulose from stationary cultures demonstrated a much higher value of Young's modulus, but a much lower value of water-holding capacity. DERWENT ABSTRACT: Pre-inoculum was prepared by transferring a single Acetobacter xylinum colony grown on SH agar culture medium into a 50 ml Erlenmeyer flask filled with liquid SH culture medium. After 48-64 hr of cultivation at 30 deg, the cellulose pellicle formed on the surface of the culture medium was either squeezed or vigorously shaken in order to remove active cells embedded in the cellulose membrane. Ten ml of the cell suspension was introduced into a 500 ml Erlenmeyer flask containing 100 ml of a fresh SH culture medium. The culture was carried out statically for 48 hr and the cell suspension derived from the synthesized cellulose pellicle was used as the inoculum for further cultures. All cultures were grown at 30 deg. The stationary cultures in either plastic trays (0.25 x 0.17 x 0.08 in) or in Erlenmeyer flasks filled with different volumes of the medium lasted for 7 days. Cultures in horizontal bioreactors equipped with disks or a roller were carried out in 2 fermentors having different volumes: 1 l and 2 l, respectively, for 7 days. Agitated cultures were carried out in 500 ml flasks on a rotary shaker at 90 rpm. The synthesized cellulose was harvested, purified by boiling it in 1 % NaOH for 2 hr, treated with 5 % acetic acid and finally thoroughly washed in tap water until the **product** became transparent(7 pages)

L6 ANSWER 15 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 2002-15081 BIOTECHDS
TI Determination of dissolved CO2 concentration andCO(2) production rate of mammalian cell suspension culture based on off-gas measurement; process **optimization** for benzylpenicillin-amidase-specific IgG1 monoclonal antibody production from hybridoma cell culture
AU FRAHM B; BLANK HC; CORNAND P; OELSSNER W; GUTH U; LANE P; MUNACK A; JOHANNSEN K; PORTNER R
CS Tech Univ Hamburg; Kurt Schwabe Inst Mess and Sensortech EV; Bundesforsch Anstalt Landwirtschaft; Tech Univ Hamburg
LO Portner R, Tech Univ Hamburg, Denickestr 15, D-21071 Hamburg, Germany
SO JOURNAL OF BIOTECHNOLOGY; (2002) 99, 2, 133-148 ISSN: 0168-1656
DT Journal
LA English
AB AUTHOR ABSTRACT - The determination of dissolved CO2 and HCO3- concentrations as well as thecarbon dioxide production rate in mammalian cell suspension culture is attracting more and more attention since the effects on major cell **properties**,such as cell growth rate, **product** quality/production rate, intracellular pHand apoptosis, have been revealed. But the determination of these **parameters** by gas analysis is complicated by the solution/dissolution of carbon dioxide in the culture medium. This means that the carbon dioxide transfer rate (CTR; which can easily be calculated from off-gas measurement) is not necessarily equal to carbon dioxide production rate (CPR). In this paper, a mathematical method to utilize off-gas measurement and culture pH for cell suspension culture is presented. The method takes pH changes, buffer and medium characteristics that effect

CO2 mass transfer into account. These calculations, based on a profound set of equations, allow the determination of the respiratory activity of the cells, as well as the determination of dissolved CO2, HCO3- and total dissolved carbonate. The method is illustrated byapplication to experimental data. The calculated dissolved CO2 concentrations are compared with measurements from an electrochemical CO2 probe. (16 pages)

L6 ANSWER 16 OF 30 BIOTECHDS on STN
AN 2000-00299 BIOTECHDS
TI Biologicals technology. Production of a carbohydrate-protein fodder **product** using potato hydrolyzates;
feedstuff production by Endomycopsis fibuligera
AU Kulinenkov D O; Mantsurova I V; Shakir I V; Panfilov V I; Manakov M N
CS Russian-Acad.Chem.Technol.
LO D.I. Mendeleev Russian Chemico-Technological Academy,, Moscow, 125190, Russia.
SO Russ.Biotechnol.; (1997) 1997, 5, 20-25
CODEN: 8381G ISSN: 1068-3682
DT Journal
LA English
AB At present one way of reducing the protein deficiency in the diet of an agricultural animal is the production of feedstuff protein via the culturing of yeasts in the hydrolyzates of plant raw materials. In this study, the conditions of preparation of the nutrient culture medium, culture **parameters** of the feedstuff yeasts on potato (Solanum tuberosum) hydrolyzate in the presence of a solid phase and the filtration **properties** of the suspensions obtained, were investigated. Cells of the Candida sp. and the Endomycopsis sp. were examined and they were cultured in 2 l (1.5 l working volume) fermentors at 30-32 deg and pH 4.5, with aeration (1 l/l), for 9 to 11 hr. The culture medium used contained (g/l) 1 KH2PO4, 0.1 K2HPO4 and 0.7 MgSO4. The results obtained demonstrated that Endomycopsis fibuligera cells may be used for the production of a carbohydrate-protein feedstuff, according to an energy-saving technology which uses filtration at the step of isolation of the finished **product**. (8 ref)

L6 ANSWER 17 OF 30 BIOTECHDS on STN
AN 1995-07895 BIOTECHDS
TI Interaction of cell culture with downstream purification: a case study;
large-scale recombinant protein purification
AU Berthold W; Kempken R
CS Thomae; Boehr.Ingelheim
LO Department of Biotechnological Production, Thomae/Boehringer Ingelheim GmbH, Birkendorfer Str. 65, 88397 Biberach at the Riss, Germany.
SO Cytotechnology; (1994) 15, 1-3, 229-42
CODEN: CYTOER ISSN: 0920-9069
DT Journal
LA English
AB Economic and fast cell separation methods were evaluated for the preparation of a **product** fluid from a large scale mammal cell culture ready for use in further ultrafiltration and chromatographic processes. Experiments were performed using a mouse hybridoma fresh cell suspension from fed-batch cultures at 80-l, 400-l and 2,000-l scale as the preharvest suspension to test the usefulness of disk stack centrifuges and tangential flow microfiltration units at a large scale. Both systems revealed outstanding prospects with regard to throughput and scale-up **properties**. However, the centrifugation did not lead to a fluid sufficiently free of particles for direct ultrafiltration or chromatography. Thus, an additional filtration step was necessary. Microfiltration led to an acceptable quality of process fluid directly. By **optimization** of process **parameters**, an effective, reproducible and robust cell separation was obtained. However, such optimal conditions are somewhat specific for a narrow range. Thus, even

the equipment functioning well with 1 type of cell would possibly not perform as well with another cell or even with the same cell under slightly different conditions. (42 ref)

L6 ANSWER 18 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1994-02019 BIOTECHDS
TI A comparison of methods for the purification of therapeutic grade monoclonal antibodies;
monoclonal antibody purification by protein-A affinity chromatography (conference abstract)
AU Cutler P; Bonnerjea J; Hill C
CS Celltech
LO Celltech, 216 Bath Road, Slough SL1 4EN, UK.
SO J.Chem.Technol.Biotechnol.; (1994) 59, 1, 110
CODEN: JCTBDC
DT Journal
LA English
AB Purification of therapeutic grade antibodies from large-scale mammalian cell culture by protein-A affinity chromatography-based processes offers a high level of purification, short cycle times and high **product** recoveries. The affinity step is relatively tolerant to small changes in operating **parameters** and is relatively easy to automate. The ionexchange chromatography-based process enables the use of well characterized, relatively low cost matrices, which are readily cleaned. A wide range of matrices (supports and functional groups) are available. the dependence of ionexchange purification on the pI of the target antibody and the impurities necessitates **optimization** of each ionexchange chromatography for each antibody. The process must be tailored to the specific physical **properties** of the **product**, e.g. sensitivity to low pH of elution from protein-A affinity matrix or the low conductivity required for ionexchange purification, ligand availability and the availability of a sensitive assay to monitor ligand leakage. (0 ref)

L6 ANSWER 19 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1993-09707 BIOTECHDS
TI 4-Aminophenyl acetate as a substrate for amperometric esterase sensors;
using acetylcholinesterase enzyme electrode (conference paper)
AU Pariente F; Hernandez L; *Lorenzo E
LO Departamento de Quimica Analitica y Analisis Instrumental, Universidad Autonoma de Madrid, Cantoblanco, 28049 Madrid, Spain.
SO Anal.Chim.Acta; (1993) 273, 1-2, 399-407
CODEN: ACACAM
DT Journal
LA English
AB 4-Aminophenyl acetate (PAPA) was used as a new substrate for the amperometric determination of esterase activities via oxidation of 4-aminophenol, the **product** of the enzyme reaction. An amperometric biosensor was developed by covalently immobilizing electric eel acetylcholinesterase (ACE, EC-3.1.1.7) on a nylon mesh membrane using glutaraldehyde and cattle serum albumin as crosslinkers. The modified membrane was then placed over a previously activated (in NaOH) glassy carbon electrode. 4-Aminophenol was detected by cyclic voltammetry and amperometry. The biosensor exhibited excellent **properties** with K'm and imax values of 6.67 mM and 10.2 uAngstroms, respectively. It also showed excellent reproducibility 92%) and long-term (over 30 days) stability when stored at 4 deg when not in use. A linear current response, proportional to the PAPA concentration, over the range 0.1 uM-0.5 mM was obtained. **Optimization** of various kinetic **parameters** of the biosensor is discussed. PAPA could also be used in the determination of ACE in whole blood and in erythrocyte concentrate. (33 ref)

L6 ANSWER 20 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

AN 1993-06159 BIOTECHDS
TI Modeling reaction and separation in the vortex flow reactor; new culture vessel application to enzyme immobilization and shear-sensitive cell culture (conference abstract)
AU Moore C M V; Cooney C L
LO Department of Chemical Engineering and Biotechnology Process Engineering Center, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
SO Abstr.Pap.Am.Chem.Soc.; (1993) 205 Meet., Pt.1, BIOT85
CODEN: ACSRAL
DT Journal
LA English
AB The new vortex flow reactor (VFR) incorporates the features necessary for continuous operation of immobilized enzyme reactions. Through the use of a secondary flow (Taylor vortex flow), the VFR provides high rates of heat and mass transfer, good mixing **properties** and the ability to process shear-sensitive cells. Applications of the VFR include adsorption of a **product** from a cell culture broth or cell homogenate, and reaction with a blood component as an extracorporeal reactor. The overall performance in the reactor as a function of kinetic, mass transfer and dispersion **parameters** was studied and correlations relating mass transfer and dispersion in vortex flow systems to operational and design **parameters** were developed. The correlations were incorporated into a comprehensive model of the VFR which allowed for the calculation of design, scale-up and **optimization** of vortex flow systems. (0 ref)

L6 ANSWER 21 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1991-11810 BIOTECHDS
TI Bench-scale co-oxidative production of stereo- and regiospecific **products** by methanotrophs; e.g. propylene oxide preparation by methanotroph fermentation in a continuous stirred tank or packed bed bubble column fermentor (conference abstract)
AU Kelley R L; Hill A H; Srivastava V J; Akin C
LO Institute of Gas Technology, Chicago, Illinois 60616, USA.
SO Abstr.Gen.Meet.Am.Soc.Microbiol.; (1991) 91 Meet., 266
DT Journal
LA English
AB The feasibility of the co-oxidative production of value-added chemicals using methanotrophs was investigated. Several of these co-oxidative **products** were evaluated for stereospecific or regiospecific **properties**. These **products** represented a range of chemical classes. These **products** were produced stereospecifically in bench-scale liquid culture fermentors for industrial evaluation. Several key operational **parameters** and conditions were determined for both a continuous stirred tank fermentor (CSTF) and a packed bed bubble column fermentor (PBF). The production phase of toxic **products** such as propylene oxide was significantly extended by intermittent addition of propylene and regeneration of the biocatalyst with methane. The performance of the CSTF and PBF for production and recovery of these **products** was described. (0 ref)

L6 ANSWER 22 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1990-12803 BIOTECHDS
TI Polymerase chain reaction - some guidelines for a successful amplification; **optimization** of conditions for DNA amplification
AU Hagen-Mann K
CS Perkin-Elmer-Cetus
LO ESSC Perkin-Elmer Cetus, Bahnhofstrasse 30, D-8011 Vaterstetten, Munich, Germany.
SO Biotech Forum Eur.; (1990) 7, 4, 313-16
DT Journal

LA English
AB The polymerase chain reaction (PCR) is an in vitro DNA amplification system which does not depend on living organisms or bacterial or viral in vivo amplification. Many improvements on the original PCR protocol have been published. The use of the Thermus aquaticus Taq DNA-polymerase (EC-2.7.7.7) instead of the Escherichia coli enzyme improved the yield, generated more specific and longer **products** and facilitated automation. The recombinant form of Taq obtained by expression of the gene in an E. coli host had the same **properties** as the native enzyme. Other factors affecting the specificity and efficiency of the PCR protocol include the PCR template, the PCR primer, the desoxynucleoside triphosphates, the buffer and the cycling **parameters**. Adjustments of these **parameters** to suit individual needs will transform an average PCR into one with excellent specificity and yield. (17 ref)

L6 ANSWER 23 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1990-05983 BIOTECHDS
TI Hydrolysis of sucrose by yeast invertase: kinetic data analysis; mathematical model describing sucrose hydrolysis by beta-D-fructofuranosidase (conference paper)
AU Stefuca V; Bales V
LO Department of Chemical Engineering, Slovak Polytechnical University, 81237 Bratislava, Czechoslovakia.
SO Prog.Biotechnol.; (1990) 6, 263-71
CODEN: PBITE3
DT Journal
LA English
AB Kinetic **properties** of yeast extracellular invertase (beta-D-fructofuranosidase, EC-3.2.1.26) were studied. The kinetics of sucrose hydrolysis was studied by both the initial rate and progress curve method. Various mathematical models differing in the **product** inhibition term were correlated with the obtained experimental data to gain the correct kinetic equation. All models considered were based on a Michaelis-Menten equation including the influence of substrate inhibition and water concentration on the reaction rate. The non-linear Gauss-Newton-Marquardt regression algorithm was used to **optimize parameter** values while the relative residual square sum was chosen as an optimum criterion. The **product** inhibition of the sucrose hydrolysis reaction was a more complex reaction than that expressed via pure competitive **product** inhibition by 1 **product**. It was proposed that models based on the conception of 2 **products** acting by different mechanisms, especially the combination of competitive and non-competitive inhibition mechanisms, give a more adequate description of the reaction course. (7 ref)

L6 ANSWER 24 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1990-13897 BIOTECHDS
TI Recent advances in the theory and practice of displacement chromatography; protein purification mathematical model (conference abstract)
AU Cramer S M
LO Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180-3590, USA.
SO J.Cell.Biochem.; (1990) Suppl.14D, 34
CODEN: JCEBD5
DT Journal
LA English
AB Displacement chromatography of biomolecules was studied, using ionexchange and reverse-phase adsorbents and metal chelate affinity chromatography systems. Affinity displacement purification of model biopolymer mixtures was carried out, and throughputs in these systems were **optimized**. The use of affinity adsorbents in the

displacement mode significantly increased the throughput and purity obtained with these selective chromatographic systems. Several novel, nontoxic polymers were synthesized for use as displacers for various adsorbent systems. The effects of critical displacer **properties** such as molecular dimensions, configuration and binding density on displacement purification of proteins were investigated. A mathematical model of displacement chromatography was developed, and included the effects of mass transport, axial dispersion and adsorption-desorption kinetics. The interplay of operating **parameters** on **product** throughput was studied, and results were compared to the predictions of the model. The model was used for **optimization** and scale-up of a significant bioseparation problem. (0 ref)

L6 ANSWER 25 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1989-05443 BIOTECHDS
TI A kinetic model for pretreated wheat straw saccharification by cellulase; application to reactor design and scale-up
AU Gonzalez G; Caminal G; de Mas C; Lopez-Santin J
LO Unitat d'Enginyeria Quimica, Facultat de Ciencies, Universitat Autonoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.
SO J.Chem.Technol.Biotechnol.; (1989) 44, 4, 275-88
CODEN: JCTBDC
DT Journal
LA English
AB Michaelis-Menten equations were used for different reaction schemes to fit experimental results obtained, working with enzyme loads between 5 and 44 IU/g pretreated wheat straw. Experiments were carried out in 500 cu cm batch reactors at 260 rpm. Reaction mixtures contained 250 cu cm cellulase (EC-3.2.1.4) solution with 12.5 g pretreated straw. Samples (1 cu cm) were removed periodically to analyze sugar contents. Kinetic **parameters** were determined by numerical fitting of experimental data using an **optimization** computer program. This program minimized the numerical values of an error function, defined as the sum of squares differences between calculated and experimental concentrations of hydrolysis **products**. A kinetic model was found by discriminating between possible reaction schemes, by analyzing the significance of each component of each possible reaction simulation. The proposed final kinetic model described the reaction process considering only macroscopic **properties** of enzyme and substrate, and was useful in reactor design and scale-up. (17 ref)

L6 ANSWER 26 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1989-06050 BIOTECHDS
TI Chromatography: 1989 report; application of various techniques to protein purification
AU Knight P
LO (Pub. Address) Nature Publishing Company, 15 East 26th Street, New York NY 10010, USA.
SO Bio/Technology; (1989) 7, 3, 243-49
CODEN: BTCHDA
DT Journal
LA English
AB Each chromatography method for protein purification can be modified to fit a particular goal, and which method is best depends on the concentration of desired **product**, physical and chemical **properties**, as well as other components in the feed. In most cases, the initial step involves ion exchange chromatography. **Optimizing** process economics involves taking into account geometric, physical, chemical and operating **parameters**. For high-pressure applications, silica has been used as medium because of its low cost and ability to withstand high pressures. Various limitations are avoided using new media, e.g. Sepharose and Sephadex polymers, designed mainly for low-pressure chromatography, widely used in process-scale work. Polymer coated silica supports offer an interesting

combination of mechanical and chemical stability. A 3 step purification process for monoclonal antibodies has been developed that performs as well as expensive affinity processes requiring many more steps. Affinity chromatography exploits biospecific interactions at the surfaces of column supports and protein molecules to purify compounds. (0 ref)

L6 ANSWER 27 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1988-10836 BIOTECHDS
TI Continuous production of maltotetraose using immobilized Pseudomonas stutzeri amylase;
glucan-1,4-alpha-maltotetraohydrolase
AU Kimura T; Ogata M; Yoshida M; Nakakuki T
CS Chiyoda; Nihon-Food
LO Engineering and Development Division, Chiyoda Corporation, 3-13, Moriya-cho, Kanagawa-ku, Yokohama 213, Japan.
SO Biotechnol.Bioeng.; (1988) 32, 5, 669-76
CODEN: BIBIAU
DT Journal
LA English
AB A continuous production process of maltotetraose was investigated by using immobilized maltotetraose (G4)-forming amylase (glucan-1,4-alpha-maltotetraohydrolase, EC-3.2.1.60) from Pseudomonas stutzeri NRRL B-3389 adsorbed onto a macroporous hydrophobic resin. The effluent from the immobilization column was then subjected to HPLC for the determination of maltotetraose (G4) content and conversion rate. The maximum reaction rate was obtained at 55 deg and the activation energy of hydrolysis by immobilized G4-forming amylase was 8.45 kcal/mol. The maltotetraose yield was greatly influenced by the flow rate of substrate solution, its concentration and the immobilized enzyme activity. The sucrose composition of the **product** was influenced by the residence time of substrate in the column because of multifunctional enzyme **properties**. It is important to **optimize** the flow rate to obtain good column performance. The newly-defined factor 'specific space velocity' was introduced to normalize the operating **parameters**. Using this factor, the immobilized enzyme reactor can be simulated and the operating dynamics can be determined. (16 ref)

L6 ANSWER 28 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1989-00968 BIOTECHDS
TI Carbohydrate metabolism in Zymomonas;
potential large-scale ethanol production by Zymomonas mobilis, review
AU Viikari L
LO Biotechnical Laboratory, Valtion Teknillinen Tutkimuskeskus, Technical Research Centre of Finland, Espoo, Finland.
SO Critical Rev.Biotechnol.; (1988) 7, 3, 237-61
DT Journal
LA English
AB Zymomonas mobilis is a potential organism for the large-scale production of ethanol. This paper reviews problems encountered in sucrose metabolism, and discusses glucose, fructose and sucrose metabolism in depth, including the enzymes involved in substrate assimilation, fermentation rate limitations, fermentation balances, byproduct formation and cell yield. Aerobic metabolism of carbohydrates is discussed and Z. mobilis strains are compared in their batch fermentations with respect to their sucrose hydrolyzing activity and subsequent ethanol formation. Tables are presented on the **properties** of Z. mobilis enzymes, metabolic rates in Zymomonas, fermentation **products** of glucose, fructose and sucrose, composition of oligomers as determined by hydrolysis with acid or invertase, distribution of byproducts in different fermentation conditions and the concentrations of individual sugars, and **products** of aerobic fermentation of glucose. In addition to **optimization** of various **parameters**, genetic methods are essential in the improvement of Z. mobilis strains for industrial utilization of sucrose-based raw materials. (111 ref)

L6 ANSWER 29 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1988-04593 BIOTECHDS
TI Special conditions for the cultivation of recombinant microorganisms; in batch and continuous culture; a review
AU Krug M; Weide H
LO Martin-Luther-Universitaet Halle, Wittenberg, WB Mikrobiologie, Bitechnikum, Am Kirchtor 1, Halle (Saale), DDR- 4020, DDR.
SO Zbl.Mikrobiol.; (1987) 142, 8, 595-604
CODEN: ZEMIDI
DT Journal
LA German
AB The use of recombinant microorganisms for batch and continuous bioprocesses is considered with regard to (i) vector construction and (ii) host-vector relationships with the aim of **optimizing** the **product** yields by appropriate variations in the genetic, physiological and biochemical characteristics of the cultures. Optimum transcription-translation efficiency is influenced by both genetic (promoter selection, ribosomal binding site design and replicon modifications) and physiological (repressor and activator concentrations and medium composition) factors. The population status is a function of genetic factors (vector construction, stability and intracellular gene concentration), physiological-biochemical **properties** of the microorganism (growth rate, intra- and extracellular **product** concentration, **product** inhibition) and **parameters** of the culture process (flow rate, substrate concentration and reactor design) which may be used in mathematical and computer models of the process. (50 ref)

L6 ANSWER 30 OF 30 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 1987-01572 BIOTECHDS
TI Rhodophyte unicells: biopolymer, physiology and production; Porphyridium aerugineum hydrocolloid production **optimization** ; potential large-scale process (conference paper)
AU Ramus J
LO Marine Laboratory and Botany Department, Duke University, Beaufort, NC 28516, USA.
SO Nova Hedwigi; (1986) 83, 51-55
DT Journal
LA English
AB Porphyridium aerugineum produces a hydrocolloid matrix which solubilizes from the cell surface to become a harvestable cell-free **product** with exploitable fluid-dynamic **properties**, e.g. high viscosities in dilute aqueous solution. Studies have been performed with regard to maximizing production of the hydrocolloid. The advantages of unicell culture over macrophyte mariculture are summarized and sufficient information exists for several species of unicellular algae to warrant the development of cost-effective protocols for large-scale production of viscoelastic biopolymers. The composition and structure of the hydrocolloid matrix have been studied in detail. Nitrogen-nutrient loading controls both cell growth and biopolymer release. P. aerugineum was cultured on a dilute salts medium in a thin-layer culture apparatus with uniform light at 25 deg. The system was operated as a chemostat for 14 days, then shifted to batch mode for comparison. Medium **parameters** have been manipulated in batch culture. It is concluded that further studies are required to **optimize** conditions for hydrocolloid production. (20 ref)

=> s analytic hierarchy
98 ANALYTIC
8 ANALYTICS
106 ANALYTIC
(ANALYTIC OR ANALYTICS)

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            81 HIERARCHY
            11 HIERARCHIES
            90 HIERARCHY
                 (HIERARCHY OR HIERARCHIES)
L7           0 ANALYTIC HIERARCHY
                 (ANALYTIC(W)HIERARCHY)

=> e nountassir/au
E1           1      NOUMURA T/AU
E2           1      NOUNO M/AU
E3           0 --> NOUNTASSIR/AU
E4           5      NOUR EL DEIN M M/AU
E5           1      NOUR EL DIN A/AU
E6           1      NOUR EL DIN A A/AU
E7           1      NOUR K/AU
E8           2      NOUR K A/AU
E9           1      NOUR M/AU
E10          3      NOUR S M/AU
E11          3      NOURAINI S/AU
E12          3      NOURBAKHSH M/AU

=> e mountassir/au
E13         20      MOUNTAIN A/AU
E14          1      MOUNTAIN L D/AU
E15          0 --> MOUNTASSIR/AU
E16          1      MOUNTEER AH/AU
E17          1      MOUNTFIELD R J/AU
E18          1      MOUNTFORD A P/AU
E19          1      MOUNTFORD D O/AU
E20          1      MOUNTFORD J/AU
E21          1      MOUNTFORD JC/AU
E22          1      MOUNTFORD P/AU
E23          7      MOUNTFORD P S/AU
E24          2      MOUNTFORD R C/AU
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